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References:

Raman, C. V. (1949) The theory of the Christiansen experiment. *Proc. Indian Acad. Sci.*, A, 29: 381-90.

Sahni, B. (1936a) Wegener's theory of continental drift in the light of Palaeobotanical evidence. *J. Indian bot. Soc.*, 15: 31-32.

Sahni, B. (1936b) The Karewas of Kashmir. *Curr. Sci.*, 5: 10-16.

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CONTENTS

STUDIES IN THE GENUS SORGHUM: II. THE CAUSE OF RESISTANCE IN *SORGHUM* TO THE INSECT PEST *ATHERIGONA INDICA* M.*

BY

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INTRODUCTION

Field studies at Siruguppa (Ponnaiya, 1951) on *Sorghum* resistance to the insect pest *Atherigona indica* M. limited the cause of resistance to a mechanical obstruction, either fibre or silica deposits, offered to the maggot at the place of cutting in the seedling stage, thus probably attributing the basic cause of resistance to the quantity and distribution of one or both of these materials.

Silicon has long been known to be present in considerable quantities in plant ash (Richardson, 1920), usually as silicon dioxide (SiO_2) popularly termed silica. The physiological role of silicon has been investigated by many workers, Knop (1861-62), Sachs (1887), Kreuzhage and Wolff (1884), Lipman (1938), Sommer (1940), and its essentiality indicated in plants like oats and rice. Further work by McColloch and Salmon (1923), Palladin (1927), Germar (1934), Briscoe (1943), Cristobal (1940), Jurado (1942) and Khanna and Ramanathan (1947) has brought about a possible correlation in plants between increased silica deposits and resistance to the attack of various parasites.

Although the absorption and deposition of silicon in tissues appear to be limited to certain species of plants and the silica pattern a constant feature for each species (Ohki, 1932) it has been found by many workers that various environmental factors such as type of soil (Wherry, 1932), pH of the soil (Whittenberger, 1945), sunlight and rate of transpiration in leaves (Germar, 1934; Miller, 1938) exert a great influence on the quantity deposited.

* Part of thesis approved for the Degree of Master of Science of the University of Madras.

The above brief review indicates that silica deposition in plants serves as an internal coat of protection against parasites which try to invade them. The formation of this silica skeleton was, therefore, investigated by the author with the aid of spodograms. The deposition of silica in relation to sunlight was also studied and the results are presented hereunder.

Material and methods

A few select *Sorghum* strains (M 47-3 and T-1 — resistant; A. S. 2095 and A. S. 1093 — susceptible) showing specific resistance or susceptibility in field trials were chosen for experimentation. Three distinct studies were made on these varieties.

1. The course of the insect was traced inside the plant and the nature of the damage investigated histologically. The attacked seedlings were spotted by the slight wilting of the fully emerged top leaf and the presence of a hatched egg on the under surface of the next lower leaf. The basal portion of the stem, about 1 cm. in length, was cut off from the rest of the plant and fixed in formalin acetic alcohol. Sections were stained in alcoholic safranin and light green (Chamberlain, 1933; Johansen, 1940).

2. Free hand sections were taken at the third, fifth and seventh leaf stages of the two types M 47-3 and A. S. 2095. The amount of lignin present in the youngest fully emerged leaf along with the growing apex at the very base of the sheath was determined by studying sections stained overnight in aqueous safranin.

3. A modified technique for preparing spodograms was evolved from the methods of Molisch (1920), Werner (1929), Ohki (1932), Herrmann (1936) and Uber (1940). Leaf sheath bits were placed flat in between two clean microscope slides and heated in a muffle furnace by gradually raising the temperature to 450°C. in the course of an hour. By constantly adjusting the rheostat, as indicated by the ammeter, the temperature was not allowed to exceed the limit and if the material was tough, it was then kept for one more hour at the same temperature to ensure complete ashing. The temperature of the furnace was then allowed to fall below 100°C., the material taken out and the top slide removed. Permanent preparations of these incinerated spodograms on microscope slides were prepared by addition of two or three drops of xylol over the ashed material followed by direct mounting in canada balsam. The slides were allowed to dry overnight at 35°C., and the resulting spodogram preserved and

examined. Silica units were measured in microns (with the help of an eye-piece micrometer standardised by a stage micrometer) and the number of silica units was counted in unit area with the help of a netz micrometer.

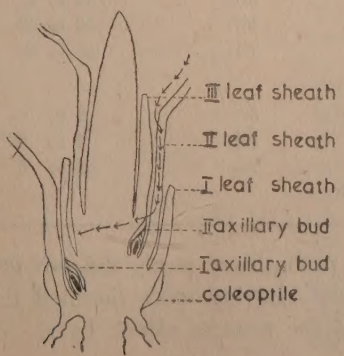
Experimental

A. *Tracing the insect injury histologically*

The route taken by the maggot after it entered the crevice between the leaf sheath and axis, was studied with the help of serial microtome sections of *Sorghum* seedlings (var. A. S. 2095) in the third leaf stage as this stage was seen to be most suitable for microtome work. (This may be due to the heavier deposits of silica in the older material).

In serial sections cut transversely, it was seen that the sections were entire to start with but soon a cavity measuring 0·12 × 0·04 mm. appeared in the middle of the second leaf sheath and extended to its very base (Plate I, Fig. 2). The comparative size of the maggot to the cavity can be judged from Plate I, Figs. 1 and 2.

In serial sections cut longitudinally, it was seen that the growing apex was separated completely from the basal portion by 0·1 mm. and the maggot was found in the lower portion of the separated apex (Plate I, Fig. 3). A diagrammatic presentation of the route taken by the maggot is shown in Text-Fig. 1. The maggot travels between the leaf sheath and the axis and lower down, probably due to the resistance offered by the clasping leaf sheath, it is unable to creep further. It then bites through the soft parenchyma cells on the dorsal side of the second leaf sheath and



Text Fig. 1.

descends making a vertical bore till it reaches the nodal end. Reaching the base, it cuts through the ventral surface of the third leaf sheath and severing the central core by making a clean horizontal cut, commences to feed on the separated central shoot as it decays.

B. *Lignified tissue in* Sorghum *as a possible factor for resistance to* Atherigona indica

In a study of transverse sections of *Sorghum* seedlings at 3rd, 5th and 7th leaf stages of the plant (see material and methods, 2), no lignified tissue was found in the growing apex. On the other hand, the youngest fully emerged leaf showed the presence of lignified tissue. The size and the number of vascular bundles and the number of layers of cells in the fibre strand of the leaf at the various stages of growth were observed and the data presented in Table I.

TABLE I.

Showing the comparative formation of lignified tissue in *Sorghum* seedlings in the resistant and susceptible varieties at zone of attack by *Atherigona indica.*

Serial No.	Description of character	Leaf stage	M. 47-3 Resistant type	A. S. 2095 Susceptible type
1.	Size of median bundle in μ	3rd	38 × 75	41 × 76
		5th	41 × 84	43 × 82
		7th	43 × 80	40 × 80
2.	Number of vascular bundles (big and small)	3rd	30 to 31	31 to 32
		5th	38 to 40	39 to 40
		7th	48 to 49	47 to 49
3.	Number of layers of cells in the fibre strand of the median bundle	3rd	3 to 4	3 to 4
		5th	4 to 5	4 to 5
		7th	4 to 5	4 to 5

Results :—This preliminary study did not give any indication on the resistant variety having more lignified tissue as compared to the susceptible at the zone of attack by *A. indica* in *Sorghum* seedlings.

C. *Distribution of silica deposits in* Sorghum

Ohki (1932) differentiated two distinct kinds of silica deposits in the Japanese *Bambusaceae* :—

(a) Dumb-bell shaped units, occurring in regular rows.

(b) Irregular in shape and occurring scattered in between regular rows of dumb-bell shaped units.

With the aid of spodograms (refer material and methods) the general pattern of silica deposits in varieties M 47-3, T-1, A. S. 2095 and A .S. 1093 of *Sorghum* were studied. It was observed that silica was deposited in most of the plant parts, the maximum quantity of the two distinct kinds of silica occurring in the leaf sheath. Dumb-bell shaped deposits were arranged in regular rows, the size of the units ranging from 12-24 $\times$ 8-12 μ. The irregularly

TABLE II.

Showing the silica deposits in the various plant parts of *Sorghum* studied by the spodogram technique.

Serial No.	Plant parts	No. of slides examined	Dumb-bell shaped silica units	Irregularly shaped units
1.	Root normal	10	Nil	Nil
	„ prop	4	Nil	Nil
2.	Leaf coleoptile	8	Nil	Nil
	„ sheath	more than 400	Present	Present after the fourth leaf stage
	„ auricle	6	Nil	Nil
	„ ligule	6	Nil	Nil
	„ midrib	20	Present	Present
	„ blade	16	Present	Present
3.	Stem apex	4	Nil	Nil
	„ node	4	Nil	Present
	„ internode	4	Nil	Present
	„ peduncle	4	Nil	Present
4.	Floral parts.			
	glume	4	Nil	Present
	awn	4	Nil	Present
	androecium	4	Nil	Nil
	gynaecium	4	Nil	Nil
	grain (tender)	—	Nil	Nil
	grain (mature)	4	Nil	Stray

shaped units lay scattered between two groups of rows of dumb-bell shaped units and their diameter ranged from 4-8 μ (Plate I, Figs. 4-9).

The distribution of the two types of deposits in the various plant organs in M 47-3 *Sorghum* is given in Table II.

Results :—No silica deposits were found in underground parts of plants and prop roots.

Silica was present in leaves, largely in the sheath, blade and midrib ; in the nodes and internodes of stems, in peduncles ; in the floral region ; in the glume and awn ; and traces in the mature grains.

The absence of silica in certain plant parts — the roots, coleoptile, ligule, auricle and the androecium — may be related to the absence of chlorophyll in these parts. Regular rows of dumb-bell shaped units occurred only in leaf parts, whereas the scattered irregular units occurred not only in leaf parts but also in the stem, peduncle, glume, awn and grain.

1. *Location of silica deposits in plant tissues*

Seventh leaf-sheath bits (M 47-3) were chosen for experimentation as this sheath yielded epidermal peels more easily than the other plant parts. The peels were removed by boiling the tissue in potassium chlorate and sulphuric acid and after washing free of the acid, spodograms were prepared out of the lower and upper epidermis.

The silica pattern was seen only on the ventral epidermis.

2. *Distribution of silica deposits in* Sorghum *leaf*

A study was made to find out more about the relationship between the vascular bundles and the silica deposits on the epidermis. In the fully grown 12th leaf of M 47-3 *Sorghum*, the tissues were thick and vascular bundles prominent. It was therefore easy to judge the distance between the bundles and epidermis with the help of a rough transverse section.

Results :—The occurrence and distribution of dumb-bell shaped silica units were closely related to the occurrence and distribution of vascular bundles in the plant. These silica units were not formed when the distance between the vascular bundles

and epidermis was considerable, while conversely they were always present, the number of rows increasing with the proximity of the epidermis to the vascular bundles. Irregularly shaped silica units were found to occur in all parts of the plant irrespective of the presence and distribution of the vascular bundles.

D. *Silica deposits in* Sorghum (*resistant and susceptible varieties*) *in relation to the attack by* Atherigona indica

Zone of resistance in Sorghum *seedlings*:—Four *Sorghum* varieties — two resistant (M 47-3 and T-1) and two susceptible (A. S. 2095 and A. S. 1093) were sown at fourteen different sowing dates at monthly intervals. The second to seventh leaf stages in seedlings were picked out at various stages of growth one from each pot, and spodograms prepared. More than 400 spodograms were examined.

Results:—Silica formation was seen clearly in the second leaf sheath as it contained very little ash to cloud the deposits. In the higher leaves, even though the ash content steadily increased it did not interfere with the silica pattern (Plate I, Figs. 4-9).

The silica pattern was seen to bear a close relationship to the epidermal pattern. Eight rows of elongated rectangular cells alternated with two to three rows of small thick walled cells. Dumb-bell shaped silica units were seen to be formed in these short cells in regular rows. The irregularly shaped silica units were formed in small rectangular cells wedged between the two narrow ends of elongated rectangular cells. They were studied separately as follows:—

Dumb-bell shaped units

(a) *Size of silica units*:—Measurements of dumb-bell shaped units were taken from spodograms of the 2nd to 7th leaf sheath in M 47-3 and A. S. 2095 *Sorghum* varieties. The results (mean of 40 measurements, 10 units from each of 4 slides selected at random) are given in Table III.

Results:—The difference in size of silica units between resistant and susceptible units on identical leaves was negligible.

Silica units in 2nd and 3rd leaves were long (23 to 24 μ). From the 4th to 7th leaf, the range was only 12 to 17 μ. Comparatively no difference in width of silica units — range 8 to 12 μ — was seen in all the leaves.

TABLE III.

Showing length, breadth and length/breadth ratio of the dumb-bell shaped units in two *Sorghum* varieties (resistant and susceptible).

Leaf sheath No.	M. 47-3 Resistant type measurements in μ			A. S. 2095 Susceptible type measuremesnts in μ		
	Length	Breadth	L/B	Length	Breadth	L/B
2	24	8	3·0	24	9	2·7
3	24	12	2·0	23	9	2·6
4	15	10	1·5	17	11	1·5
5	14	10	1·4	16	10	1·6
6	13	9	1·4	13	9	1·4
7	12	8	1·5	13	8	1·6

(b) *Number of rows of silica units* :—The number of rows occurring in each group was counted in all the four varieties of *Sorghum* and it was seen that there was no difference between the resistant and susceptible varieties in the numbers of rows on identical leaves.

Irregularly shaped silica units :—Their earliest appearance in spodograms was in the third leaf of M 47-3 and T-1 types of *Sorghum*, where stray units were observed. Their origin was traced to the small rectangular cells lying wedged between two narrow ends of thin-walled elongated cells of the epidermal layer of leaf sheaths. Because of their fixed position with respect to elongated cells, the distribution of these silica units appeared to be governed by the length of the elongated cells which varied considerably in the second to the seventh leaves.

Table IV presents the average length and breadth of the elongated cell and the size and distribution of the irregularly shaped silica units in the various leaves of M 47-3.

Results :—It was generally seen that the elongated epidermal cells were longest in the second and third leaf sheaths and any reduction in length of these cells was correlatable with increased

TABLE IV.

Showing the length and breadth of the long cells and size and distribution of the irregular silica units (Resistant variety M47-3).

Number of leaf sheath	Elongated epidermal cell		Irregularly shaped silica unit	
	Length in μ	Breadth in μ	No. of units in 155 μ square	Size in μ
2	265 ± 36	15	Not formed	Not formed
3	206 ± 40	18	Stray	4 × 14
4	120 ± 15	17	3 ± 0·9	8 × 17
5	72 ± 10	16	7 ± 2·4	10 × 18
6	54 ± 12	16	8 ± 2·2	17 diameter
7	48 ± 5	14	11 ± 3·0	17 diameter

number of irregularly shaped silica units. Tables V and VI give the distribution and size of irregularly shaped units in the other three *Sorghum* types.

TABLE V.

Showing the distribution of irregularly shaped silica units in an unit area of 155 μ square in the resistant and susceptible varieties in the various leaf sheaths.

Leaf sheath number	Resistant types		Susceptible types	
	M. 47-3	T-1	A. S. 2095	A. S. 1093
2	Not formed	Not formed	Not formed	Not formed
3	Stray	Stray	Not formed	Not formed
4	3	4	Not formed	Not formed
5	7	6	Stray	Stray
6	8	9	9	8
7	11	11	12	11

TABLE VI.

Showing the size in μ of irregularly shaped silica units in the resistant and susceptible varieties in the various leaf sheaths.

Leaf sheath number	Resistant types		Susceptible types	
	M. 47-3	T-1	A. S. 2095	A. S. 1093
2	Not formed	Not formed	Not formed	Not formed
3	4 × 14	3 × 14	Not formed	Not formed
4	8 × 17	5 × 15	Not formed	Not formed
5	10 × 18	10 × 51	5 × 14	6 × 14
6	17 diameter	15 diameter	10 × 14	12 × 14
7	17 diameter	16 diameter	16 diameter	14 diameter

Results:—The irregularly shaped silica units were well formed in the fourth leaf sheath of the resistant varieties (Plate I, Fig. 4), whereas in the susceptible variety, its uniform formation appeared only in the sixth leaf sheath (Plate I, Fig. 9). The maximum size and spherical shape of the units were, however, reached only at the sixth and seventh leaf stages respectively in the resistant and susceptible varieties.

These results have a bearing on the field studies recorded earlier by the author (Ponnaiya, 1951, Table VIII). It was emphasized then, that in the two resistant varieties studied, the fourth leaf had a higher percentage of escapes than the identical leaf stage of the susceptible variety. It was also observed that the escapes of the fourth leaf of the resistant variety were almost equal to the escapes observed at the seventh leaf stage of the susceptible variety.

Thus, the earlier deposition of silica in the small cells wedged between two elongated epidermal cells of the leaf sheaths of *Sorghum* seedlings in certain varieties seems to be an inherent character and is possibly the basic cause of resistance to the attack by *Atherigona indica*.

E. *Influence of sunlight on silica deposits*

It has been recorded by Germar (1934) and Miller (1938) that transpiration and bright illumination have a direct influence on silica deposition. Hence the following experiments were performed to investigate the influence of sunlight on silica deposition.

A comparison of the spodograms of leaves of resistant *Sorghum* plants (variety M 47-3) located in shade, sunlight and total darkness, leaves collected on the day of emergence and three days after emergence showed that:

1. Plants in the shade, on the day of emergence of all the six leaf stages had poorly formed dumb-bell shaped silica deposits and no irregularly shaped silica units. However, material gathered three days after emergence (in the shade) showed well formed silica deposits comparable to the material gathered on the day of emergence in open sunlight.

2. Leaves in plants exposed to three days sunlight showed no more well formed silica units than on the first day. It is, therefore, concluded that lack of direct sunlight only delays formation of silica deposit and that under normal sunlight, the deposits appear to be fully formed on the day of leaf emergence itself and further exposure to sunlight shows no further visible increase.

3. Total darkness not only retarded the formation of silica but reduced the number of dumb-bell shaped silica units formed.

F. *Source of silica deposits in* Sorghum

M 47-3 *Sorghum* seedlings were grown in Crone's nutrient solution free from silicon (Miller, 1938) and spodograms of the basal portion of the leaf sheaths from the various leaves on the day of their emergence were prepared and examined. The first six seedling leaves were subjected to this study.

Silica deposits in trace quantity of delicate outlines of dumb-bell shaped units were observed at the base of leaf sheaths in the first three leaves and the other parts were free from it. From the 4th to 6th leaves, no silica was observed indicating that silica in the soil is essential for accumulation in *Sorghum*. According to Raleigh (1945), even air-borne silica can contaminate the cuture solution and this may explain the stray deposits of silica in the three leaves.

G. *Process of silica deposition in* Sorghum

Newly formed young leaves of *Sorghum* were found to be free from silica and hence it was thought desirable to know at what age of the leaf, after emergence, silica was deposited.

It was observed that it takes three days for the tender leaf to emerge fully from the leaf sheath, after the first appearance of the tip of the blade.

The fifth leaf (M 47-3) was examined for silica deposits at this stage (three days before full emergence) and a spodogram was prepared daily for three days till the tender leaf emerged fully. The next spodogram of the material was taken one month after emergence.

The above study indicated that silica deposits were formed in the leaves of *Sorghum* only two days prior to emergence. But the rate of deposition was rather quick and was complete on the day the leaf fully emerged. No additional deposits took place even after one month's time.

The physical properties of these dumb-bell shaped units were studied with the aid of polarized light and no extinction was observed between crossed nicols when the stage was rotated. Possibly these 'crystals' are so minute that the characteristic birefringence of silica could not be observed at ordinary magnifications. This indicates the probability of these units being micro-crystals of colloidal silica of the class of borosilicates.

Discussion

The fly *Atherigona indica* was found by the author to be a major pest attacking *Sorghum* — a fact which has not hitherto been recorded. In a critical study between resistant (M 47-3) and susceptible (A. S. 2095) varieties, leaf by leaf, the attack was seen to be suddenly checked at the fourth leaf stage in the resistant variety and in the 6th leaf in the susceptible, the attack completely stopping at the eighth leaf stage in both varieties. Further, it was seen that the maggot was able to cut the growing apex in 100% of the seedlings at the youngest leaf stage (second leaf), the percentage getting reduced gradually in the higher leaf stages, to no attack at the eighth leaf stage even in the susceptible varieties. The presence of large deposits of silica suggested of its being a possible factor for resistance. The spodogram technique revealed that there was practically no difference between the resistant and

susceptible varieties in the formation of dumb-bell shaped silica units on identical leaves. The irregularly shaped units, however, were found to be almost absent in the 2nd and 3rd leaves of both the resistant and susceptible varieties. A uniform deposit of this type of unit was found to occur in the 4th leaf of the resistant and on the sixth leaf of the susceptible variety. The main difference between the resistant and susceptible varieties was, therefore, in the comparatively earlier formation of the irregularly shaped silica units in the former which accounted for the resistance. These observations fit into data on the field occurrence when the fly is capable of attacking the plants, whether susceptible or resistant, only up to four weeks and not later, since there is no difference in the formation of the irregularly shaped silica units after the fourth week (8th leaf stage) in both the varieties.

Summary

In *Sorghum,* lignified tissue was not a factor for resistance to the insect pest *Atherigona indica,* as there was no difference in the number and size of vascular bundles and the number of cells in the fibre strands between the resistant and susceptible varieties. Two distinct kinds of silica deposits, the dumb-bell shaped silica units occurring in regular rows and the irregularly shaped silica units lying between groups of rows of dumb-bell shaped units, bore a close relationship to the epidermal pattern.

The occurrence and position of dumb-bell shaped silica units were uniform and identical in both the resistant and susceptible varieties and this structure was, therefore, not the probable cause for resistance. The irregularly shaped silica units though absent in the second and third leaves of both the varieties, yet were well formed from the fourth leaf onwards in the resistant varieties and from the sixth leaf in the susceptible varieties. The earlier formation of these silica units in resistant varieties, therefore, indicated the basic cause of resistance to *Atherigona indica* M.

Plants grown in silica-free nutrient solution were found to have only traces of these deposits in the first three leaves, probably originating from the seeds or from contaminated air. Deposition of silica in the leaf sheath commenced only three days before emergence of the leaf but was rapid and complete on the day of emergence of the leaf. Diffused light retarded the formation of silica deposits while total absence of light not only retarded but also reduced the quantity deposited.

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REFERENCES

Briscoe, H. V. A. (1943) Some new properties of inorganic dusts. *J. R. Soc. Arts.* 91: 593-607 (cited from *Rev. appl. Ent.,* Series A, 1944, 32: 38).

Chamberlain, C. J. (1933) *Methods in Plant Histology.* The University of Chicago Press—Chicago, Illinois, 1933. p. 416.

* Cristobal, U. L. (1940) Conservacion de les granos almacenados El. "nuevo" metodo de los polvos silicicos. (The preservation of stored grain. The "new" method of siliceous dusts). *Bol. Lab. zool. Agric. Fac. Agron. Univ. nac. La Plata* 5: 8 (cited from *Rev. appl. Ent.,* Series A, 1942, 30: 30).

* Germar, B. (1934). Some functions of silicic acid in cereals with special reference to resistance to mildew, *Pflanzenernähr. Düng. u. Bodenk.,* A. 35: 102-15, *Chem. Abst.* 29: 1461. (cited from Miller, 1938).

Herrmann, F. (1936) Erweiterung des verfahren der Schnittveraschung Differenzeirung der anorganischen Struktur gesunder und Kranker *Haut zeits Wiss Mikr,* 52: 257-75. (cited from Uber, 1940).

Johansen, D. A. (1940) *Plant Microtechnique.* McGraw-Hill Book Company, Inc. New York and London, 1940 pp. 523.

* Jurado, A. J. R. (1942) La Sillice en la conservacón de les cereals. (Silica for the conservation of cereals). *Rev. Argent. Agron.,* 9: 1-18. Buenos Aires. (cited from *Rev. appl. Ent.,* Series A, 1944, 32: 339).

Khanna, K. L. and Ramanathan, K. R. (1947) Studies on the association of plant characters and pest incidence. *Proc. Nat. Inst. Sci., India,* 8: 327.

* Knop, W. (1861-62) Quantitativ-analytische Arbeiten über den Ernährungs process der Pflanzen. *Landw. Versuchs Stat.,* 3: 295-324; 4: 173-85. (cited from Miller, 1938).

* Kreuzhage, C. and Wolff, E. (1884) Bedeutung der Kieselsaure fur die Entwicklung der Hafer Pflanze, *Landw. Versuchs Stat.,* 30: 161-97. (cited from Miller, 1938).

Lipman, C. B. (1938) Importance of silicon, aluminum and chlorine for higher plants. *Soil Sci.,* 45: 189-98.

McColloch, J. W. and Salmon, S. C. (1923) The resistance of wheat to the Hessian fly. A progress report. *Jour. econ. Ent.,* 16:293-98.

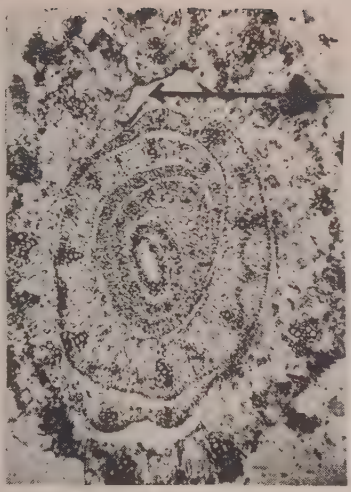
Miller, E. C. (1938) *Plant Physiology.* McGraw-Hill Book Company, Inc., New York and London. p. 1201.

* Molisch, H. (1920) Aschenbild und Pflanzenverwandtschaft *Sitzungsber. Akad. Wiss. Wien. Math.-Nat. Kl.* 1, 129: 261-94. (cited from Uber, 1940).

Ohki, K. (1932) On the systematic importance of spodograms in the leaves of the Japanese *Bambusaceae. Jour. Faculty Sci. Imp. Univ., Tokyo.,* Section III. Botany, 4: 1-129.

Palladin, V. I. (1927) *Plant Physiology.* Eng. trans. Livingston. (cited from Miller, 1938).

Fig. 1



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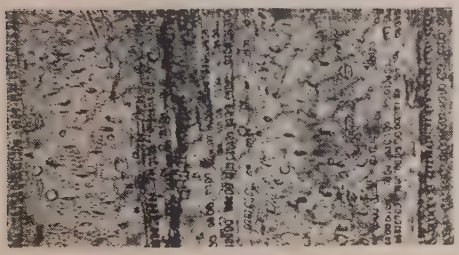

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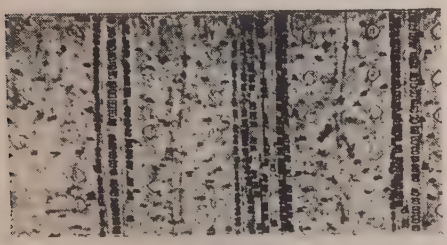

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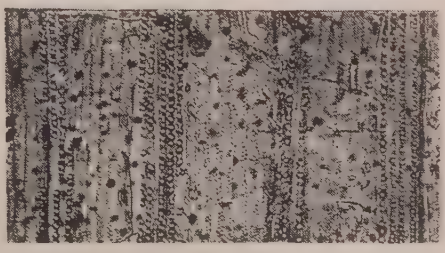

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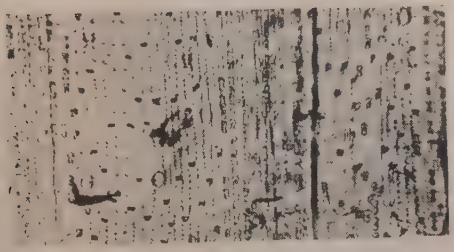

Fig. 6

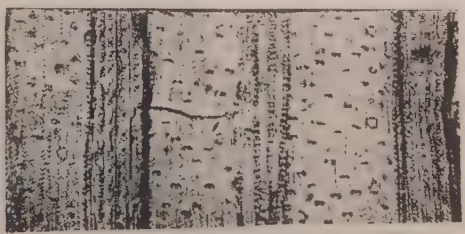

Fig. 9

Fig. 1. Photomicrograph showing the maggot of *A. indica* (× 30). Fig. 2. Photomicrograph of transverse section of attacked plants just above region of attack. Note the cavity in the parenchyma (× 50). Fig. 3. Photomicrograph of longitudinal section of the same material. Arrow indicates route taken by maggot (× 50). Figs. 4 to 6: Photomicrographs of spodograms of the 4th, 5th and 6th leaf sheaths respectively of resistant variety (M 47-3). Figs. 7 to 9. Photomicrographs of spodograms of the 4th, 5th and 6th leaf sheaths respectively of susceptible variety (A. S. 2095).

Ponnaiya, B. W. X. (1951) Studies in the genus *Sorghum*: I Field observations on *Sorghum* resistance to the insect pest *Atherigona indica* M. J. *Madras Univ.* B. 21: 96-118.

Raleigh, G. J. (1945) Silicon in plant growth. *Soil sci.* 60: 133-35.

* Richardson, W. D. (1920) The ash of dune plants. *Science*, 51: 546-51. (cited from Miller, 1938).

* Sachs, J. (1887) *Lectures on the Physiology of Plants.* Eng. ed., Clarendon Press. (cited from Miller, 1938).

Sommer, A. L. (1940) Mineral nutrition of plants. *Ann. Rev. Biochem.*, 9: 487-88.

Uber, F. M. (1940) Microincineration and ash analysis. *Bot. Rev.*, 6: 204-26.

* Werner, O. B. (1929) Ein neuer Apparat zur Gewinnung von Pflanzen für Aschenbildbestimmungen *Mikrochemie.*, 7: 110-15. (cited from Uber, 1940).

* Wherry, E. T. (1932) Ecological Studies of Serpentine barren plants. I. ash composition. *Proc. Pennsylvania Acad. Sci.*, 6: 32-38. (cited from Whittenberger, 1945).

Whittenberger, R. T. (1945) Silicon absorption by rye and sunflower. *Amer. J. Bot.*, 32: 539-49.

* Not seen in original.

AN INTERESTING INSTANCE OF "VIVIPAROUS" GERMINATION IN *CARICA PAPAYA* L.

BY

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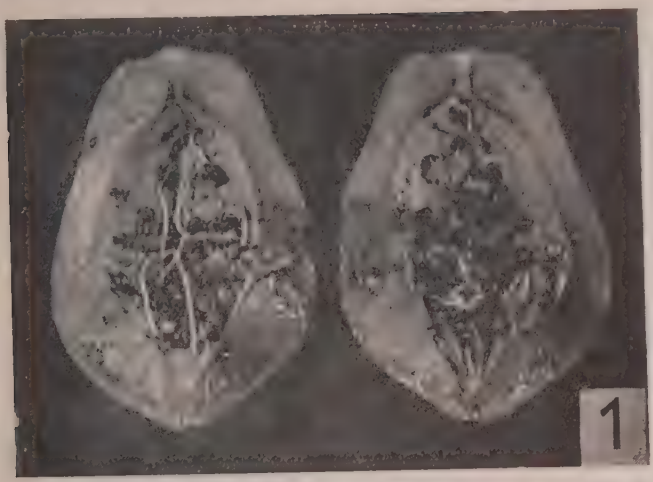
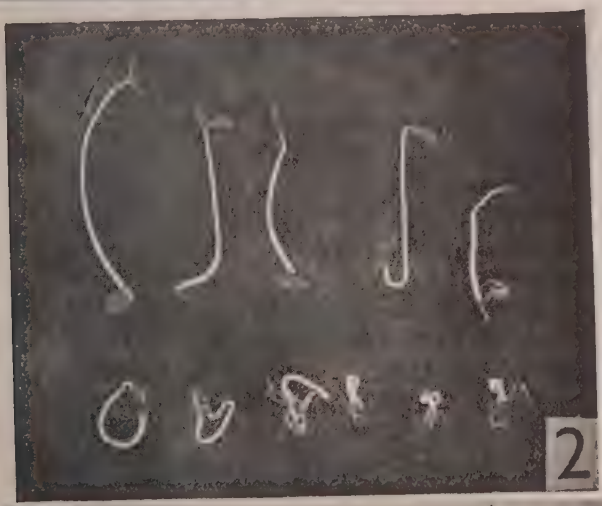
An instance of "viviparous" germination in *Carica papaya* was brought to my attention sometime ago. Germination of seed within the fruit of this species seems to be a common occurrence; but more than mere germination, the seedlings in this case exhibited a special feature which, I think, is worth recording.

The fruit was normal looking, fully ripened and when cut open showed a number of sprouted seeds (Fig. 1). In the case of the papaya seed there does not seem to be any dormant period and it is, therefore, likely that the seeds were in a condition fit for germination. The exact factor contributing to the sprouting of the seeds within the fruit, however, was not known. Different stages of germination and growth were observed (Fig. 2), but the most characteristic feature of the seedlings was that their cotyledons were distinctly green in colour, contrary to what can usually be expected in seedlings grown in a dark enclosed space. Some plants and plant parts seem to be exceptions to the general rule in that they become green in the dark,[1] but such a feature does not occur in the case of the normal papaya seed germinated in the absence of light. It may also be of interest to note here that a similar case of germination of seeds within stored pumpkins, the cotyledons of the seedlings being distinctly pale-green, was recently recorded.[2]

One of the requisites for the formation of chlorophyll is light. The development of chlorophyll in the cotyledons of the seedlings within the fruit (i.e., virtually in the dark) is, therefore, remarkable. It is rather difficult to explain precisely how the healthy green colour could have developed. Probably, owing to some physiological disturbance the content of "protochlorophyll", a pigment which develops without the aid of light and changes photochemically into chlorophyll upon exposure to light,[3] was so highly developed in the cotyledons that even a sudden exposure to light was sufficient for the formation of a healthy green colour.





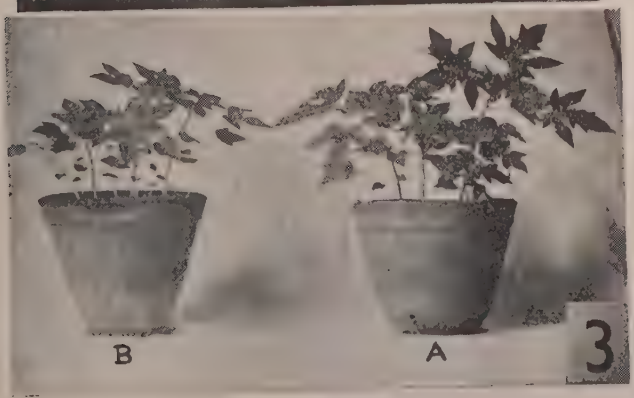


Fig. 1. Papaya fruit in section showing germinated and ungerminated seeds. Fig. 2. Seeds showing different stages of germination. Fig. 3. Ten-weeks-old plants. A = from normal seeds. B = from sprouted seeds.

The pulp of the papaya fruit exhibiting this abnormal feature was sweet unlike that of the apple with viviparous seedlings,[4] which was insipid suggesting that the apple seedlings might have utilised the sugars for their growth.

As a matter of interest, the germinated seeds and normal ungerminated ones were planted separately in pots at the same time and their subsequent growth was observed. Although the former had an initial start, the latter showed a slightly better performance at the end of ten weeks (Fig. 3).

My grateful thanks are due to Prof. T. S. Sadasivan for kindly placing the material at my hands.

REFERENCES

1. Miller, E. C., "Plant Physiology", McGraw-Hill Book Co., New York and London, 1931.

2. Klemm, M., *NachrBl. dtsch. PflSchDienst*, 1948, 2; 216-17 (*Hort. Abst.*, 20; abst. 1679).

3. Eyster, W. H., *Science*, 1928, *68*; 569-70.

4. Mani, V. K. S., *Curr. Sci.*, 1947, 87; 321.

SOMATIC CHROMOSOMES IN THE ACANTHACEAE

BY

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Introduction

Acanthaceae is a large family of Angiosperms, including 220 genera and 2000 species (Rendle, 1925). The chromosome numbers of only 16 species belonging to five genera, appear to have been recorded so far. Some of these, are meiotic and a few mitotic counts of Sugiura (1936). Mitotic counts of five species of *Ruellia* have been recorded by Bowden (1940 and 1945). Chromosome numbers of *Thunbergia grandiflora* by Janaki Ammal, *Acanthus spinosus* by Drahowzal, two species of *Justicia* and *Hygrophila spinosa* by Sugiura are also available in the Chromosome Atlas of Darlington and Janaki Ammal (1945). To this meagre list can now be added, the chromosome numbers of 28 species belonging to 18 divergent genera. One species, *Barleria cristata* includes four separate varieties. Of these 28 species, 17 are wild in nature, although some among them have been brought under domestication; the rest of the species are mainly of horticultural interest. The present study is undoubtedly limited considering the size of the family. The conclusions drawn therefore, may be regarded as tentative.

Materials and Methods

Root-tips of seedlings and roots developed from cuttings specially planted, were fixed in Navashin's fluid, after pre-fixation in Carnoy's fluid for 30 seconds, as well as in the modified Bouin's fluid of Sass (Johansen, 1940). The fixing was done in different parts of the day. Materials fixed in the morning gave satisfactory results. Sections from 8 to 14 microns were cut and stained in Haidenhain's iron alum Haematoxylin. Sections of spp. of *Barleria* and a few others were also stained in Newton's iodine gentain violet. Good results were obtained from both the fixatives and stains. In many cases Bouin's fluid definitely excelled Navashin's in giving better staining of the chromosomes with Haematoxylin. Sketches were drawn with the help of the camera lucida at an approximate magnification of 2200 diameters.

Observations

The sequence of the genera in the present list, is after the classification of Gamble (1924).

1. *Thunbergia.* Two species of this genus were studied. In *Thunbergia alata* (Figs. 1 and 1a). 2n = 18, and this number agrees with Sugiura's (1936) meiotic count. n = 9. The chromosomes are large in relation with the other species in the present study, with the exception of *Barleria* spp. (Figs. 15 to 20a). Of the nine pairs of chromosomes (Fig. 1a), seven pairs are definitely longer and have median or slightly sub-median centromeres. In *Thunbergia erecta* (Fig. 2) where 2n = 52, the chromosomes are only about one half of the larger chromosomes in *T. alata* (Figs. 1 and 1a) and do not seem to have intercalary constrictions.

2. *Meyenia erecta.* In this species, which is allied to *Thunbergia* spp. 2n = 52 (Fig. 3). The karyotype corresponds closely with that of *Thunbergia erecta.* (Fig. 2).

3. *Acanthus ilicifolius.* Fig. 4 is a metaphase plate of this species, with 48 almost uniform chromosomes. (The species appears to be an unstable polyploid, judging from the occurrence of metaphase plates with 44 chromosomes and the casting out of a few chromosomes into the cytoplasm in many cells in the same root-tip sections).

4. *Dyschoriste littoralis* (*Calophanes littoralis*). The chromosomes of this species are slightly smaller than in the previous species (Fig. 4). Fig. 5 is a metaphase plate with 28 uniform chromosomes, some of which have median constrictions.

5. *Eranthemum.* The chromosomes in the five species of this genus studied, are relatively the smallest in the present list, corresponding more or less with those of *Andrographis.* (Figs. 12 and 13) and *Belloperone* (Fig. 31). In *Eranthemum rosea* 2n = 30 (Fig. 6); in *Eranthemum variegatum* 2n = 34 (Fig. 7); in *Eranthemum metalicum* 2n = 34 (Fig. 8); in *Eranthemum longiflorum* 2n = 38 (Fig. 9); *In Eranthemum cinnabaricum,* while the metaphase plate of one root-tip shows 2n = 34 (Fig. 10) in another root-tip 2n = 38 (Fig. 10a). A comparison of the figures 10 and 10a, shows that in Fig. 10a, some of the chromosomes of the set appear in parallel pairs, perhaps suggestive of somatic pairing.

6. *Strobilanthes* sp. In this unidentified species, where 2n = 28 (Fig. 11), the chromosomes are alike and some with

FIGS. 1 TO 16a.

1. Metaphase plate of *Thunbergia alata*. 1a. Idiogram of the same. 2. Metaphase plate of *Thunbergia erecta*. 3. Metaphase plate of *Meyenia erecta*. 4. Metaphase plate of *Acanthus ilicifolius*. 5. Metaphase plate of *Calophanes littoralis*. 6. Metaphase plate of *Eranthemum rosea*. 7. Metaphase plate of *E. variegatum*. 8. Metaphase plate of *E. metalicum*. 9. Metaphase plate of *E. longiflorum*. 10 and 10a. Metaphase plates of *A. cinnabaricum*. 11. Metaphase plate of *Strobilanthes* sp. 12. Metaphase plate of *Andrographis paniculata*. 13. Metaphase plate of *A. echioides*. 14. Metaphase plate of *Crossandra undulifolia*. 15. Metaphase plate of *Barleria prionotis*. 15a. Idiogram of the same. 16. Metaphase plate of *B. buxifolia*. 16a. Idiogram of the same.

apparently median constrictions. In size they correspond to those of *Calophanes littoralis* (Fig. 5).

7. *Andrographis.* The chromosomes in the two species of this genus studied are again relatively very small. In *Andrographis paniculata,* $2n = 28$ (Fig. 12); in *Andrographis echioides* $2n = 38$ (Fig. 13). One can observe in the metaphase plate of *A. echioides* (Fig. 13), some of the chromosomes in parallel pairs, perhaps suggestive of somatic pairing.

8. *Crossandra undulifolia.* Fig. 14 is the metaphase plate, with 20 rod-shaped chromosomes, some of them with median constrictions. In point of size, the chromosomes come close to those of *Calophanes littoralis,* (Fig. 5) and *Strobilanthes* sp. (Fig. 11).

9. *Barleria.* The chromosomes of this genus are relatively the largest in the present study, with the exception of *Thunbergia alata* (Figs. 1 and 1a). In *Barleria prionotis,* $2n = 30$ (Figs. 15 and 15a). Size differences, which are confirmed during the study of meiosis of this species are apparent in the members of the complement. Approximately 6 pairs are shorter than the remaining 9 pairs. Many of the chromosomes seem to have median or sub-median constructions. In *Barleria buxifolia,* which is allied to *B. prionotis* $2n = 38$ (Figs. 16 and 16a). The idiogram (Fig. 16a) shows, that approximately 8 pairs of chromosomes are relatively shorter than the remaining 11 pairs. A correspondence in the morphology of many of the chromosomes of this species with those of *B. prionotis* (Fig. 15a) is also obvious.

Barleria cristata, is a very variable species in nature (Gamble, 1924), with white, pink, blue and striped flowers and divergent leaf form and size. The chromosome numbers of the varieties are also different. In the white-flowered variety $2n = 34$ (Figs. 17 and 17a); in the pink-flowered variety $2n = 36$ (Figs. 18 and 18a); in the striped-flowered variety $2n = 38$ (Figs. 19 and 19a) and in the blue-flowered variety $2n = 40$ (Figs. 20 and 20a). While in the white, pink and striped varieties (Figs. 17 to 19a) both long and short chromosomes are present in each of the complements, in the blue variety (Figs. 20 and 20a) the chromosomes are either medium or short, long chromosomes being absent when compared with the sister varieties. The karyotype of the striped variety (Fig. 19 and 19a), seems to have an individuality of its own. The disparity between the longer and shorter chromosomes is not so sharply marked as in the remaining varieties.

10. *Asystasia.* Of the two species of this genus studied, in *Asystasia travancorica* $2n = 28$ (Fig. 21); the chromosomes of the metaphase plate are similar. *Asystasia coromandeliana* is described variable in nature (Gamble, 1924), with white, blue or purple and yellow flowers. The root-tips of the yellow flowered variety were studied. There is variability of chromosome number in the different metaphase plates of the same root-tip sections, associated with the loss of some chromosomes into the cytoplasm in some cells. The highest number of chromosomes counted was 52 (Fig. 22). The chromosomes are similar and in size nearly equal to those of *Acanthus ilicifolius* (Fig. 4).

11. *Aphelandra cristata.* The metaphase plate (Fig. 23) of this species shows 68 similar and rod-shaped chromosomes.

12. *Sanchezia, parvibracteata.* Fig. 24 is the metaphase plate of this species with 80 chromosomes, the highest number recorded in the present study. The chromosomes are similar and correspond in size to those of *Aphelandra cristata* (Fig. 23).

13. *Rungia repens.* In this species where $2n = 20$ (Figs. 25 and 25a), all the chromosomes are similar and apparently have median constrictions. The idiogram (Fig. 25a) bears a striking resemblance to that of *Peristrophe bicalyculata* (Fig. 30a).

14. *Ecbollium Linneanum.* var. *laetevirens.* In this species $2n = 28$ (Fig. 26) and the chromosomes are nearly comparable in size to those of *Justicia* spp. (Figs. 27 and 28).

15. *Justicia.* The chromosomes of the two species of the genus studied are relatively very small. In *Justicia betonica* $2n = 28$ (Fig. 27); in *Justicia Gendarussa* $2n = 32$ (Fig. 28).

16. *Rhinacanthus montana.* (Fig. 29) is the metaphase plate with 32 relatively large chromosomes. The idiogram (Fig. 29a) shows slight inequality among the chromosomes of the complement. The last two pairs of chromosomes appear shorter than the rest.

17. *Peristrophe bicalyculata.* In this species, where $2n = 20$ (Figs. 30 and 30a), the chromosomes appear strongly resembling those of *Rungia repens* (Figs. 25 and 25a). The idiogram (Fig. 30a) also corresponds to that of the latter species (Fig. 25a).

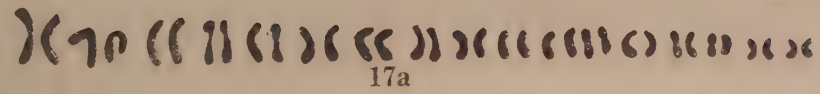
18. *Bellopperone nemerosa.* Fig. 31 is the metaphase plate of this species, with 62 similar chromosomes. In point of size, the chromosomes are among the smallest in the present study.

17

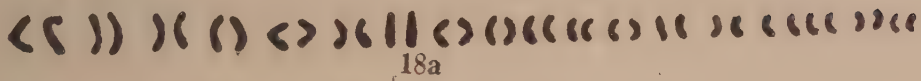
18

19

20



17a



18a

19a

20a

FIGS. 17 TO 20a.

17. Metaphase plate of *Barleria cristata*. (white-flowered). 17a. Idiogram of the same. 18. Metaphase plate of *B. cristata* (pink-flowered). 18a. Idiogram of the same. 19. Metaphase plate of *B. cristata* (striped flowered). 19a. Idiogram of the same. 20. Metaphase plate of *Barleria cristata* (blue-flowered). 20a. Idiogram of the same.

Discussion

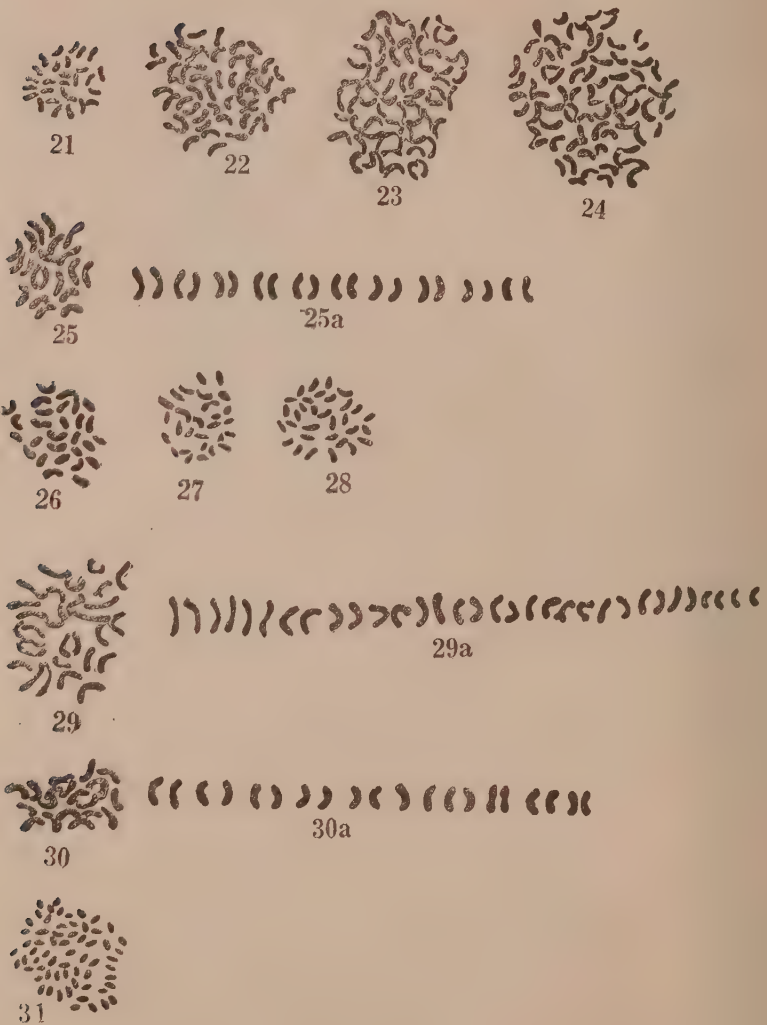
Aneuoploidy. Aneuoploid chromosome numbers have been recorded in a number of genera and families of Angiosperms, Heilborn, (1924) in the genus *Carex*: Manton, (1932) in the Cruciferea's: Venkatasubban, (1944) in the genus *Tecoma*: Venkatasubban, (1945 and 1946) in the Palmae and Scitamineae respectively: Moore, (1947) in the Loganiaceae and Bowden, (1948) in the Anonaceae. Coming to the Acanthaceae, aneuoploidy has been previously recorded in the genus *Ruellia* (Sugiura, 1936) and in the genus *Thunbergia* (Sugiura, 1936 and Janaki Ammal, 1945). Differences in the number of chromosomes in one and the same species have also been recorded. Thus, according to Bowden (1940a) in *Ruellia tuberosa* $2n = 34$, but according to Sugiura (1936) $2n = 32$. In *Acanthus spinosus* $2n = 112$ according to Sugiura, (cited in the Chromosome Atlas, 1945) but according to Drahowzal (cited in the Chromosome Atlas, 1945) $2n = 80$. In *Hygrophila spinosa*, $2n = 24$ according to Sugiura (cited in the Chromosome Atlas 1945) but according to Venkatasubban (1944) $n = 16$. As regards *Ruellia tuberosa*, the occurrence of two numbers, 32 and 34, is confirmed during the study of meiosis of this species (Narayanan, unpublished). In the 12 plates of Metaphase I of this species, in 10, the number of bivalents was 16, while in the remaining two, it was 17. No univalents were observed. The occurrence of aneuoploid chromosome numbers in several of the species recorded here, appears thus to be in harmony with the previous chromosome counts.

Aneuopolidy is said to arise by the duplication of some of the chromosomes of a set, or by their transverse septation or fragmentation. Both the possibilities occurring in meiosis has been suggested by Heilborn (1924) in the genus *Carex*. Navashin (1930) has observed, two halves of one chromosome passing to one pole in *Crepis tectorum*, causing aneuoploidy in one of the two daughter nuclei. Darlington (1937) has stated that both fragmentation and fusion of chromosomes have occurred, causing disparity of their number in the different species of *Fritillaria*. Venkatasubban (1944), on the basis of measurements, has pointed out, that fragmentation is the cause of aneuoploidy in the genus *Tecoma*.

Both duplication and fragmentation of the chromosomes appear to be the cause of aneuoploidy in the Acanthaceae. For example, the relationship between *Thunbergia alata*, with $2n = 18$ (Figs. 1 and 1a) and *Thunbergia erecta*, with $2n = 52$ (Fig. 2)

cannot be established, unless one assumes, that, the chromosomes of the long and medianly or sub-medianly constricted type which make the majority of the complement in *T. alata* (Fig. 1a) undergo median septation, followed by duplication of some of the chromosomes. The basis for such an inference, is the higher number ($2n = 52$) of shorter chromosomes, which appear without intercalary constrictions in *T. erecta* (Fig. 2) and presence of 7 pairs of long and medianly or sub-medianly constricted chromosomes in the total complement of 18 in *T. alata* (Figs. 1 and 1a).

Both duplication and fragmentation appear again to be evident in the genus *Barleria*. A comparison of the metaphase plates and idiograms of the white variety of *Barleria cristata* (Figs. 17 and 17a) with the blue variety (Figs. 20 and 20a) of the same species, shows clearly, the absence of long chromosomes and the higher total number of chromosomes ($2n = 40$) in the blue variety (Fig. 20a). The presence of a few pairs of longer and apparently medianly constricted chromosomes in the white variety, with $2n = 34$ (Fig. 17a) is also obvious. Transverse septation of the longer chromosomes of the white variety (Fig. 17a) resulting in an increase in the total number of chromosomes in the blue variety (Fig. 20a) seems to be the cause of the different chromosome numbers in these two examples. The pink variety of the same species, with $2n = 36$ (Figs. 18 and 18a) has a higher number of shorter chromosomes, when compared with the white variety (Figs. 17 and 17a) and this *prima facie* appears to be the result of duplication of one pair of the shorter chromosomes of the white variety. The duplication of short chromosomes of a complement owing to the non-separation of the two halves at mitosis, leading to unequal chromosome numbers in the different nuclei of the same plant has been pointed out by Darlington (1937). The karyotype of the striped variety with $2n = 38$ (Figs. 19 and 19a), has an individuality of its own, when compared with the karyotypes of the sister varieties (Figs. 17 and 17a, 18 and 18a and 20 and 20a). In this variety (Figs. 19 and 19a) there are more pairs of longer chromosomes, and even the shorter chromosomes are bigger in relation with similar ones of the other varieties (Figs. 17 and 17a, 18 and 18a and 20 and 20a). It seems difficult to relate the striped variety by a gross comparison of its karyotype with the other three varieties. The higher chromosome number may be the result of duplication of some of the chromosomes of the species. As regards disparity in chromosome size, a probable explanation is, that it is the effect of fixitive-stain reaction (Sharp, 1934).



FIGS. 21 TO 31.

21. Metaphase plate of *Asystasia travancorica*. 22. Metaphase plate of *A. coromandeliana*. 23. Metaphase plate of *Aphelandra cristata*. 24. Metaphase plate of *Sanchezia parvibracteata*. 25. Metaphase plate of *Rungia repens*. 25a. Idiogram of the same. 26. Metaphase plate of *Ecbollium Linneanum*. 27. Metaphase plate of *Justicia betonica*. 28. Metaphase plate of *J. Gendarussa*. 29. Metaphase plate of *Rhincanthus montana*. 29a. Idiogram of the same. 30. Metaphase plate of *Peristrophe bicalyculata*. 30a. Idiogram of the same. 31. Metaphase plate of *Belloperone nemerosa*.

Barleria prionotis (Figs. 15 and 15a) and *Barleria buxifolia* (Figs. 16 and 16a) are taxonomically near each other. The idiogram of *B. buxifolia* (Fig. 16a) with 2n = 38, shows an obvious correspondence in a number of chromosome pairs, with that of *B. prionotis* (Fig. 15a) with 2n = 30. The numerical disparity of the chromosomes between the two species should be then the result of duplication of some of the chromosomes inherent in the genus.

Chromosome duplication leading to speciation is also evident in the genus *Andrographis* (Figs. 12 and 13). In *Andrographis paniculata* 2n = 28 (Fig. 12); in *Andrographis echioides* 2n = 38 (Fig. 13). The chromosomes of the two species are similar. The higher number in *A. echioides* (Fig. 13) along with the parallelism of some of the chromosomes in the metaphase plate, points to such a conclusion. One is drawn to a similar conclusion in the genus *Eranthemum* (Figs. 6 to 10a), in the five species of which, three chromosome numbers viz., 30, 34 and 38 are met with. The chromosomes are almost uniform in the different species. Another example of this type, is *Justicia betonica with* 2n = 28 and *Justicia Gendarussa* with 2n = 32 (Figs. 27 and 28).

Karyotypes: Karyotypes are defined on the basis of chromosome number, size and morphology. As the chromosome numbers of only 44 species belonging to 23 genera (inclusive of the present study) are known, in the family, it will not be useful to proceed with the study of karyotypes on the basis of chromosome numbers alone. On the basis of chromosome size and morphology, however, some useful conclusions can be drawn.

One can observe for instance, the wide disparity in size of the chromosomes occurring in the different genera of the family. In *Thunbergia alata* (Figs. 1 and 1a) and *Barleria spp.* (Figs. 15 to 20a) both of which are however taxonomically distant, are found the largest chromosomes, while the smallest chromosomes are found in *Eranthemum* (Figs. 6 and 10a), *Andrographis* (Figs. 12 and 13) *Justicia* (Figs. 27 and 28) and *Belloperone nemerosa* (Fig. 31). This wide difference, occurring as it does within members of the same alliance, strikes one as extreme. Another observation is the dissimilarity of the karyotypes between taxonomically or morphologically related plants, and the similarity of it between unrelated or distantly related genera. Thus, for example, *Rungia repens* with 2n = 20, (Fig. 25 & 25a) is taxonomically nearer to *Echollium Linneanum* with 2n = 28 (Fig. 26) and *Justicia* spp. with 2n = 28 and 32 (Figs. 27 and 28, than to *Peristrophe bicalyculata* (Figs. 30 and

30a). Yet one observes the striking similarity in the karyotypes including chromosome number between *R. repens* (Figs. 25 and 25a) and *P. bicalyculata* (Figs. 30 and 30a). On the other hand no karyological relationship seems possible between *R. repens* (Figs. 25 and 25a) and *E. Linneanum* and *Justicia* spp. (Figs. 26, 27 and 28). *Crossandra undulifolia* with 2n = 20, is far removed taxonomically, from either *R. repens* (Figs. 25 and 25a) or *P. bicalyculata* (Figs. 30 and 30a), both with 20 somatic chromsomes. A similarity of karyotypes of taxonomically less closely related plants is furnished by the following examples :—*Acanthus ilicifolius* (Fig. 4) with 2n = 48. and *Asystasia coromandeliana* (Fig. 22) with 2n = 52; *Dyschoriste littoralis* (Fig. 5) with 2n = 28 and *Strobilanthes* sp. (Fig. 11) with 2n = 28. The resemblance of the examples in each pair on the basis of morphology is limited; but the chromosomes resemble, both in number and structure.

Two noteworthy features about the karyology of the family are thus, the disparate chromosome numbers and the disparate chromosome sizes. Babcock (1934), Sharp (1934) and Venkatasubban (1945) have pointed out to the possibility of such divergence in number, size and morphology of the chromosomes among plants belonging to the same natural alliances. Babcock (1934) mentions about diversity of chromosome sizes even in species of the same genus and cautions against the drawing of cytotaxonomical relationships on the basis of the karyotypes from a small fraction of the species in a family or genus. Sharp (1934) again has observed generally, that cultural methods and fixitives are likely to alter chromosome size and volume. One may conclude, in the light of the present study of the Acanthaceae, that evolution and species formation in this family has proceeded not only by change in number but also in the size of the chromosomes. Further studies in the family are required to confirm this suggestion.

Summary

The somatic chromosomes of 28 species of 18 divergent genera of the Acanthaceae have been described.

Both aneuploid chromosome numbers and disparate chromosome sizes are observed even in this limited study.

Evolution and speciation in the family appears to have proceeded by a change in number and size of the chromosomes.

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LITERATURE CITED

Babcock, E. B. (1934) Basic Chromosome numbers in Plants, with special reference to the Compositae. *New Phytol., 33:* 386-388.

Bowden, W. M. (1940) Diploidy, Polyploidy and winter hardiness relationships in higher plants. *Amer. J. Bot., 27,* 357-371.

Bowden, W. M. (1945) A list of chromosome numbers in higher plants I, *Amer. J. Bot., 32,* 81-92.

Bowden, W. M. (1948) Chromosome numbers in the Anonaceae. *Amer. J. Bot., 35,* 7.

Darlington, C. D. (1937) *Recent Advances in Cytology,* pp. 63, 77, Churchill, London.

Darlington, C. D. and Janaki Ammal, E. K. (1945) *Chromosome Atlas of cultivated plants,* p. 258, George Allen and Unwin, London.

Gamble, J. S. (1924) *Flora of the Presidency of Madras,* pp. 1002-1006, 1060 and 1063. Published under the authority of the Secretary of State in Council.

Heilborn, O. (1924) Chromosome numbers and dimensions, species formation and phylogeny in the genus *Carex, Hereditas,* 5, 129-216.

Johansen, D. A. (1940) *Plant Microtechnique,* p. 47, McGraw Hill, N. Y. and London.

Manton, I. (1932) Introduction to the general cytology of the Crucifereae. *Ann. Bot., 46,* 509-556.

Moore, R. J. (1947) Cytotaxonomical studies in the Loganiaceae, *Amer. J. Bot., 34,* 527-538.

Navashin, M. (1930) Unbalanced Somatic chromosomal variation in *Crepis. Univ. Calif. Pub. Agri. Sci. 6,* 95-106 (cited in Darlington's Recent Advances in Cytology).

Rendle, A. B. (1923) *The classification of Flowering Plants. Vol. 2,* 544, Cambridge University Press.

Sharp, L. W. (1934) *Introduction to Cytology.* pp. 126-131. McGraw Hill, N. Y. and London.

Sugiura, T. (1936) Studies on the chromosome numbers in higher plants with special reference to cytokinesis I. *Cytologia, 7.,* 544-595.

Venkatasubban, K. R. (1944) *Cytological studies in Bignoniaceae,* pp. 141-205, Annamalai University Publication, Annamalai Nagar.

Venkatasubban, K. R. (1945) Cytological studies in Palmae, Pt. I., *Proc. Indian Acad. Sci. 22,* 194-207.

Venkatasubban, K. R. (1946) A preliminary survey of chromosome numbers in Scitamineae of Bentham and Hooker *Proc. Indian Acad. Sci. 23,* 281-300.

NUCLEOLAR CONDITIONS IN MEIOSIS OF *BARLERIA PRIONOTIS*

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Introduction

In the literature concerning the nucleolus, mention has been made about the duality of its constitution. Selim (1931), in his study of meiosis of *Oryza sativa,* describes two kinds of nucleoli, primary and secondary, the latter appearing to be budded out of the former in early prophase. The same author (1931) also cites Ludford and deMol in support of his contention. Zirkle (1931) speaks about the differentiation of the nucleolus into two parts on the evidence of its vacuolate structure. Doutreligne (1933) points out to the differentiation in prochromosomal plants, of the nucleolus into a clear central region and a darker peripheral region. Vanderlyn (1948) in his review, speaks of the frequent picture of dichotomy of nucleolar substance, the discontinuous elements contained within the continuous, as one or several vacuoles or as several refractive crystalline bodies. During the study of meiosis and mitosis of *Barleria prionotis* and other species of the Acanthaceae, all of which are prochromosomal plants, clear evidence of such a dichotomy of nucleolar substance is encountered.

Materials and Methods

Flower buds, fixed in Navashin's fluid, after prefixation in Carnoy's fluid, and also in Sass's modified Bouin's fluid (Johansen, 1940) were sectioned at 12 to 16 microns in thickness. Material fixed in Bouin's fluid was stained only in Haidenhain's iron alum Haematoxylin and that in Navashin's in both this stain and Newton's iodine gentian violet. A simplified schedule, which gave satisfactory results for the latter was adopted. Sketches were drawn with the help of the Camera lucida at an approximate magnification of 2500 diameters.

Observations

The pachytene stage of the pollen mother cells (P. M. Cs) is characteristic in showing in the vast majority of cases a bouquet

arrangement of the chromosome threads in relation with the large nucleolus (Fig. 1). A semi-bouquet arrangement (Fig. 2) is less frequent, while the absence of it with only two bivalents attached to the nucleolus, the remaining lying scattered in the nuclear sap is rarest (Fig. 3), being spotted only in one P.M.C. of the several flower bud sections examined. The typical bouquet arrangement of the pachytene threads in relation with the nucleolus (Fig. 1) is also met within the P.M.Cs of *Justicia betonica* and *Ruellia tuberosa,* two other species of the Acanthaceae (Narayanan, unpublished). Such a configuration finds a striking correspondence with that recorded by Rao (1930) in *Cyanotis cristata.* At diakinesis in the majority of the P.M.Cs., the nucleolus appears considerably reduced in size, although different in chromaticity in the different nuclei (Figs. 4 and 5). In some of the P.M.Cs. it appears not to have undergone any appreciable reduction and it is in these (Figs. 6 and 7) that it shows a clear differentiation into an inner clear region and a peripheral darker region. In no case, was the nucleolus seen to persist at metaphase, while it has disappeared even at diakinesis in a few P.M.Cs.

The two regions of the nucleolus normally do not appear bodily separated from each other throughout meiosis. In sections of one flower bud in which the P.M.Cs. are in the pachytene stage, however, the separation appears obvious. Figs. 8, 9 and 10 show this abnormal phenomenon. In Fig. 10, a lightly chromatic and in Fig. 9 a deeply chromatic spherical body appears to come out of the nucleolus, like a vescicle and one gets the impression of the inner region of the nucleolus escaping from the outer region through an opening in the latter. In Fig. 8, there appears a chromatic band at the junction between the nucleolus and the vescicle escaping from inside it. At the same time, the pachytene threads appear collapsed. The separation of the two parts of the nucleolus appear thus correlated to the influence of the fixative. There is no separation of the nucleolar components when pachytene is normal; but separation of the two is accompanied by collapse of the pachytene threads. It is interesting to note, that neither collapse nor distortion of the nucleolus or the body coming out of it takes place; both retain their spherical form. Such a phenomenon does not seem to have been recorded previously.

The situation in mitosis of the root-tip cells of the same species is slightly different. Here, the nucleolus in the resting and prophase nucleus, shows the presence of one to four small vacuoles, as in Fig. 12, a phenomenon which is also present in other species

of the Acanthaceae. In *Thunbergia alata,* however, two conditions are met with in the nuclei of the same root-tip sections. In some (Fig. 11), the nucleolus is differentiated into a darker outer region and a colourless inner region as in the P.M.Cs. of *Barleria prionotis* (Figs. 6 and 7); in others (Fig. 12), the nucleolus is vacuolate as in the root-tip nuclei of *B. prionotis.* As in meiosis, there is no persistence of the nucleolus at somatic metaphase; there is diminution and disappearance of it closely associated with the chromosomes during phophase.

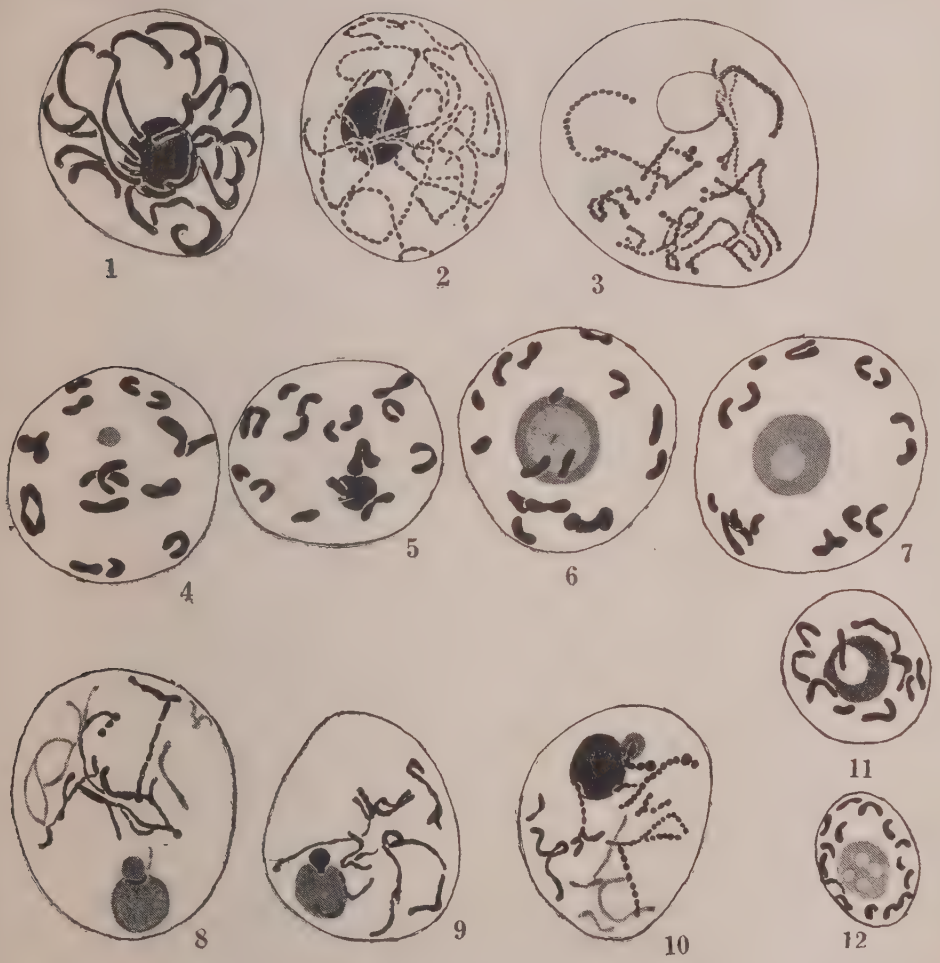
Discussion

Zirkle's (1931) argument, that a vacuolate nucleolus is indicative of its composition by two substances of different refractive indexes, holds good in the somatic nucleolus of the present species. Doutreligne's (1933) observation, regarding the nucleolus of prochromosomal nuclei also holds good in the root-tip nuclei of *Thunbergia alata* and those of the P.M.Cs. of *Barleria prionotis.* The surmise of deMol, that the inner part of the nucleolus perhaps escapes through a pore in the outer part is actually observed in the present species, despite the fact that one could not in the light of recent literature lend support to his conclusions regarding the role of the nucleolus. Selim (1931) has recorded and figured the presence of two nucleoli in the pollen mother cells of *Oryza sativa,* in a good number of cases, as though it is a natural and not an induced phenomenon. In the present species the bodily separation of the two nucleolar components in meiosis appears clearly to be an induction of the fixitive, because it is accompanied by the collapse of the pachytene threads. The conclusions of Selim (1931) like the conclusions of deMol do not again conform to the present conceptions regarding the role of the nucleolus. The phenomenon recorded here, however, appears useful in the interpretation of the nucleolar structure. The purpose of the present paper is not a discussion, either of the role of the nucleolus or its chemical composition. There is considerable literature on the subject, which needs no recounting here. Evidence from morphology appears to point out that the nucleolus, at least in prochromosomal plants, consists of two components, the bodily separation of which is possible under certain conditions.

Summary

Nucleolar structure has been described in the pollen mother cells in the pachytene and diakinesis stages, of *Barleria prionotis.*

The nucleolus consists of two parts, which sometimes become bodily separated. The inner colourless part appears to come out of the darker outer part through an opening in the latter.



Meiosis in *Barleria prionotis*.

1. Pachytene stage showing typical bouquet. 2. Pachytene stage showing semi-bouquet. 3. Pachytene stage without bouquet. 4. and 5. Diakinesis in two different P.M.Cs.; note the reduced and differently chromatic nucleoli in the two. 6 and 7. Diakinesis in two other P.M.Cs., showing clear differentiation of the nucleolus into two regions. 8, 9 and 10. Pachytene collapse accompanied by the vescicle-like escape of the inner part of the nucleolus through the outer part. A chromatic band at the junction of the two bodies is apparent in 8. 11. Prophase nucleus in the root-tip of *Thunbergia alata*, showing differentiation of the nucleolus into two regions. 12. Prophase nucleus in another cell of the same species, showing vacuolate nucleolus.

REFERENCES

Doutreligne, J. (1933) Chromosomes et nucleolus dens less noyaux, du type euchromocentrique. *Cellule,* 1933, *T. 42,* 31-100.

Johansen, D. A. (1940) *Plant microtechnique.* p. 47. McGraw Hill Book Co. N. Y. and London.

Rao, N. S. (1930) On reduction division in pollen mother cells of *Cyanotis cristata. J. Indian bot. Soc. 9, 2 and 3.* 79-113.

Selim, A. G. (1931) A cytological study of *Oryza sativa, Cytologia.* 2. 1-26.

Vanderlyn, L. (1948) Somatic mitosis in the root-tip of *Allium cepa, Bot. Rev. 5,* 270-318.

Zirkle C. (1931) Nucleoli of the root-tip and cambium of *Pinus strobus, Cytologia, 2,* 85-105.

NOTE ON CHROMOSOME FRAGMENTATION IN MEIOSIS OF *BARLERIA PRIONOTIS*

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Fragmentation or transverse breakages overtaking chromosomes, have long been known. Induction of such changes by x-radiation or thermal treatment in plant and animal cells or tissues, is also well known. Our knowledge of the time and place of such and other structural changes of chromosomes, occurring under natural conditions, is however relatively meagre. Sharp (1934) has observed, that there is the possibility of breakages following contact and entanglements of the prophasic chromosome threads in meiosis. One definite mode of fragmentation observed by Belar (1929), is that, in which a small portion of one chromosome is torn away from its synaptic mate in the first meiotic anaphase. A similar process has been strongly suggested in a *Zea* sporocyte observed by Randolph (Sharp, 1934). In the meiosis of the Pollen Mother Cells (P.M.Cs) of *Barleria prionotis,* there is clear evidence of such a chromosome irregularity, during the first anaphase.

Fig. 1

Fig. 2

Figs. 1 and 2 represent meiotic anaphase I, in lateral view of P.M.Cs. belonging to the same flower bud sections. In both, there is one pair of relatively longer chromosomes showing belated separation, when compared with the rest of the chromosomes. In Fig. 1, the process of separation appears nearing completion, the

distal ends of the chromosomes appearing to be drawn into a fine thread-like connection, which appears different from the spindle fibres. In Fig. 2 the separation is completed and one can observe a small fragment lying loose between the ends of the separated chromosomes. The configuration of the chromosomes in both the cases appears to correspond to the description given by Darlington (1937). According to Darlington (1937) bivalents which have the greatest length of chromatids to pull apart, lag behind the rest during meiotic anaphase. Such chromosomes are seen at anaphase with their two chromatids separated throughout their length. The ends of the chromosomes may also become drawn out into a fine thread which may be invisible. The two figures here appear to fit in with the description of Darlington (1937). The chromosomes that lag in relation with the rest, are comparatively longer. Their chromatids are separated throughout their length. The attenuation of the distal ends during their separation is found in Fig. 1. The small rod-like chromatic body seen in the spindle between the separated chromosomes in Fig. 2 appears prima facie to be the distal end of the bivalent that has undergone no disjunction, and is left behind as a fragment. If the configuration represented here is interpreted as a chromosome bridge, then it does not resemble chromosome bridges as figured in *Chorthippus* (Darlington 1937), *Rheo discolour* (Bhaduri, 1942) and *Tradescantia* (Bhaduri, 1942). There is neither a gap in a chromosome bridge, nor are the chromatids separated throughout their length, as seen in the present case. It seems therefore, that the interpretation of fragmentation in the present case is fairly conclusive. What happens to the fragment is a different problem. Presumably it becomes lost into the cytoplasm.

Material and method. Flower buds fixed in Navashin's after pre-fixation in Carnoy's were sectioned at a thickness of 18 microns and stained in Newton's iodine gentian violet. Sketches were drawn at an approximate magnification of 4800 diameters.

REFERENCES

Belar, K. (1929) Mechanism of mitosis. *Roux's Arch. Entw. 118,* 359-484 (cited in Sharp's Introduction to Cytology).

Bhaduri, P. N. (1942). Cytological analysis of structural hybridity in *Rheo Discolour* Hance. *J. Genet. 44,* 73-85.

Bhaduri, P. N. (1942) Application of new technique to Cytological re-investigation of the genus *Tradescantia, J. Genet, 44,* 87-126.

Darlington, C. D. (1937) *Recent Advances in Cytology,* pp. 114, 116 and 270. Churchill, London.

Sharp, L. W. (1934) *Introduction to Cytology,* pp. 315-316. McGràw Hill Book Co., N.Y. and London.

BIONOMICS AND EARLY DEVELOPMENT OF GLOSSOGOBIUS GIURIS (HAMILTON)[1]

BY

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Introduction

Our knowledge of the bionomics, breeding habits and development of the Indian Gobies is still very meagre, even though the contributions of Willey (1911), Bhattacharya (1916), Raj (1916), Aiyar (1935), Jones (1937) and Panikkar and Aiyar (1939), among others[2] have furnished valuable information on the breeding and development of species like *Gobius astericola, Glossogobius giuris, Acentrogobius neilli,* A. *viridipunctatus,* etc. A detailed account of the early development of *Glossogobius giuris,* together with notes on its bionomics and breeding habits, is furnished in the present paper.

Bionomics

The largest of the true Gobies and growing to over a foot in length *G. giuris* is a valuable food fish with a wide range of distribution. Locally called "*Uluvai*", it is one of the common and best-known freshwater fishes of Madras, occurring in ponds, ditches, lakes and rivers. With flesh of fair flavour, but devoid of fine bones, it is esteemed as a food fish in several localities and adds considerably to the bulk of marketable fish, owing to its fair size and abundance. It is reported to reach its maximum size in fresh water lakes. The largest specimen in the present collection, obtained from the Chetput Fish Farm, Madras, measured 12·4 inches in total length. Hardly any information is available now about its growth in different habitat.

1. Paper read before the 34th Indian Science Congress, New Delhi, 1947, and published with the kind permission of the Director of Fisheries, Madras.

2. An account of the larval and post-larval development of *Gobiopterus chuno* by Pillai and Sarojini (*Proc. Nat. Inst. Sci. Ind.,* XVI, (3), pp. 181-187) and a similar account of the embryonic development of the same species by Chaudhuri (*Proc. Nat. Inst. Sci. Ind.,* XVII (4) 1951) have recently been published.

G. giuris thrives in salt water as well as fresh water habitats. It is a bottom feeder, lurking among weeds and quickly darting at objects of prey. Colouration of the body is often so remarkably adapted that it easily merges with that of the surroundings. The large wide mouth, provided with prominent sharp teeth, clearly indicates its predacious habit. Systematic analyses of the stomach contents of specimens from different fresh water habitats were made to ascertain their exact feeding habits. Predatory throughout life, marked piscivorous tendency, often amounting to cannibalism, is evident in the larger specimens. Young individuals are predominently insectivorous and they frequent and stealthily explore the shallow, weedy margins of ponds for aquatic insects and larvae. Vegetable portion of the diet was almost invariably insignificant. Mookerjee *et al* (1947) found Crustaceans forming 65% of the feed in specimens 80 to 130 mm. long. Adult specimens of *Chela* spp., *Barbus* spp., *Rasbora daniconius* and *Amblypharyngodon mola* have been frequently met with in the stomach contents of larger specimens. Even fingerlings, 70 to 80 mm. long, were found to be piscivorous. "Uluvai" thus thrives in weedy waters containing an abundance of aquatic insects and carp minnows. Because of the piscivorous tendencies, it is generally removed from carp nurseries, though complete eradication is extremely difficult without dewatering the pond.

Breeding Habits

The most interesting feature about this Goby is that it breeds both in fresh water as well as in salt water. Certain salt water fishes like *Chanos chanos, Megalops cyprinoides, Lates calcarifer, Mugil* spp. etc., are known to be capable of easy acclimatisation to fresh water, but they are not known to breed in that environment (Job and Chacko, 1947). *G. giuris,* however, shows greater adaptation to extreme environmental conditions, and closely resembles in this respect, the Indian Cichlids, the Pearlspot (*Etroplus suratensis*) and the Orange-Chromide (*E. maculatus*), both of which breed freely in salt water as well as in fresh water (Job, *et al* 1948; Alikunhi, 1948).

Raj (loc. cit.) considers that " the typical *G. giuris* is an exclusively fresh water form " and that " the variety *kokius* is confined to backwaters and the sea." This variety, however, does not appear to have been recognised by later workers (Hora, 1936, Koumans, 1941). According to Hora (loc. cit.) *G. giuris* is one of the few Gobies that migrate to the sea for breeding purposes. Herre

(quoted by Guillermo and Villadolid, 1939) states that "in Nature, the eggs are attached to the underneath of rocks near or in the mouth of rivers where they are influenced by the tides". It is reported to breed from May to July in Ceylon (Willey, loc. cit.) and from September to December in Madras (Raj, loc. cit.; Panikkar and Aiyar, loc. cit). Observations made at the Chetput Fish Farm, Madras, confirm that the species freely breeds in fresh water, without any migration to salt water for this purpose. It is also found that while intensive breeding takes place in ponds from September to December, breeding on a limited scale takes place even as early as April. Thus, the species is probably a perennial spawner, the peak period of spawning perhaps coinciding with the rainy months.

Sexual maturity is attained rather early in life, specimens about 85 mm. and over in total length, having been found with ripe gonads. Guillermo and Villadolid (loc. cit.) state that when sexually mature *G. giuris* is about 22 to 144 mm. long. The ripe ovary is bright yellow in colour in the fresh condition and the outline of the ovaries is fairly visible through the thin ventral abdominal wall. Nothing is known about the fecundity of the species. With increase in size, fecundity will also increase; a patch of laid eggs from one of the farm ponds numbered approximately 75,000.

The fish is known to attach eggs to the under surface of submerged hard objects. Willey (loc. cit.) found the eggs attached to tiles, to the undersurface of coconut leaf and stalk and also inside an iron piping. Occasionally, eggs are found in the deserted burrows of crabs (Raj, loc. cit.). In the Chetput Farm, eggs have been found naturally deposited on the underside of tiles and wooden planks and also inside small tins. Successful attempts were also made to induce the Goby to deposit eggs on particular objects specially provided for the purpose. A small wooden plank was kept on two bricks, submerged in water near the weedy margin of the pond and a weight was placed above to prevent the plank from floating up. On the second day when the plank was lifted up and examined, a healthy patch of fertilized eggs was seen attached to the under surface. This could be repeated several times and in most cases, by the second or the third day after keeping the plank in position, spawn could be obtained.

The exact process by which the eggs are fertilized and deposited on the under surface of the plank is not known. The parents are reported to guard the spawn (Raj, loc. cit.) and our observations also indicate the same. If a net is suddenly cast over the place

where the plank is kept for inducing egg-laying, in most cases the parent Gobies are caught. Both the parents are probably engaged in the care of spawn. If the parents are once scared off from the spawn, they do not appear to immediately return to their charge, with the result that some other fish readily eat away the entire spawn. On several occasions the submerged planks were just lifted up and then immediately replaced after noting the stage of development of the eggs. In all such cases, however, a second examination, even within an hour, revealed that the spawn had been eaten off by some predator. The parents, however, do not appear to have the tendency to eat their own spawn. On one occasion a patch of eggs deposited on the under surface of a plank was removed along with one of the parent fishes to a conditioning box kept in the same pond. Subsequent observations showed that the fish confined itself to the region where the eggs were deposited and it successfully nursed the spawn to hatching, even though it was starving during the period. Under natural conditions, the parent fishes might constantly be aerating the spawn by gently stirring the water with their fins; for, if the parents are removed and the spawn mass placed in pond water without any subsequent disturbance, almost invariably excessive mortality results in the developing embryos. Parental care appears to cease with the hatching of the eggs.

Development

Ovarian Egg: (Fig. 1) : The ripe ovarian egg is elongated, about 1·26 mm. long (ranging from 1·2 mm. to 1·3 mm.); slightly swollen at one end (diameter : 0·36 mm.) and narrower at the other (diameter: 0·24 mm.). A number of long, extremely slender, hair-like processes are found attached to the narrower end of the egg. These filaments are adhesive and when the eggs are kept in a dish of water they stick to the bottom by means of these filaments. The vitelline membrane closely adheres to the egg surface. When teased out the ovum appears pale white in colour.

(a) Embryonic Development

Fertilized Egg: (Fig. 2) : The fertilized egg is almost rounded or oval in shape, about 0·66 mm. in length and 0·48 mm. to 0·50 mm. in diameter. The changes that take place soon after fertilization could not be observed, but the earliest stage obtained is, probably, not more than an hour after fertilization. The vitelline membrane has swollen into a conspicuous, elongated, finger-like capsule or

envelope which is attached to the substratum at one end, through the adhesive filaments that have now coalesced into a short stalk-like structure. This attached end of the tube is narrow, while the other end is broader, though smoothly tapering to the tip and contains the developing embryo. As the spawn is generally attached to the undersurface of objects, the egg tubes hang down, with the egg at the terminal portion (Plate 1a). The length of these tubes varies from 3·0 mm. to 6·0 mm., variation being found even in the same batch of eggs.

Several oil globules of different size are found in the fertilized egg. When the egg capsule is hanging down, these oil globules gradually migrate to the upper region of the yolk mass.

Cleavage and Blastoderm Formation : Segmentation is partial and the blastoderm is formed at the upper portion of the yolk mass, in about 6 hours after fertilization. It is a translucent, lightly granular area, easily distinguishable from the yellow, opaque yolk mass.

Invasion of Yolk : (Fig. 3). Within the next two hours about two-thirds of the yolk surface is invaded by the blastoderm cells. The oil globules have shifted to one side from their former median position. In another two hours, the yolk is almost completely covered over by the blastoderm cells.

Embryo, 10 hours after fertilization: (Fig. 4). Embryonic ridge is prominent, but, head and tail ends are hardly distinguishable.

Embryo, 12 hours after fertilization: Head end of the embryo is differentiated towards the upper pole of the egg, away from the hanging tip of the capsule. Oil globules are getting coalesced and are less numerous than in the previous stage. The embryo is slightly elongated and measures about 0·81 mm. to 0·84 mm. in length. Rudiments of the optic cups are just visible. 6-7 myotomes have also appeared.

Embryo, 15 hours after fertilization: (Fig. 5). Embryo has further elongated and is about 1·0 mm. long. 10-11 myotomes have appeared. Yolk-sac has become oblong. There are one or two large and three or four small oil globules. In some, only two large oil globules are present and these are situated near the anterior end of the yolk mass. The tail end is elongated and is free from the yolk. Embryonic fin fold has appeared around the tail. Optic cups are transparent. The auditory rudiments are visible as clear areas, far behind the optic cups. Head end of the embryo is still turned away from the tip of the capsule.

Embryo, 24 hours after fertilization: It now measures about 1·8 mm. in length. Yolk is getting steadily absorbed. The oil globules have already coalesced into a single, large one. The tail reaches the distal end of the capsule and is often curved forwards. The embryo has begun executing movements. Circulation of blood has been set up. Eyes are not pigmented. Two concretions have appeared in each of the auditory vesicles. Dorsal and ventral embryonic fin folds are well formed. 3-4 chromatophores are present on the yolk-sac ventrally. Likewise, near the anal region also a few scattered chromatophores have appeared along the mid-ventral line.

Embryo, 32 hours after fertilization: (Fig. 6). About 24 myotomes have differentiated. Eyes begin to get pigmented. A complete change in the orientation of the embryo within the egg capsule takes place, with the result that the head is now directed towards the distal closed end of the capsule (Plate 1b). With this reversal of position the elongating tail gets ample space for free movement and it is no longer curved or curled.

Embryo, 42 hours after fertilization: Pectoral rudiments have appeared. Eyes are dark when viewed against a white background. Yolk-sac is relatively small, with the single oil globule. Pulsations of the heart are regular, 120 to 124 times per minute. Some chromatophores have appeared near the eyes. Chromatophores along the mid-ventral line form a more or less continuous row. Total length of the embryo is now about 2·22 mm.

Embryo, 48 hours after fertilization: (Fig. 7). It has further elongated to about 2·32 mm. Eyes are completely pigmented. Pectoral fin buds are distinct on either side, at the level of the anterior margin of the yolk-sac. Rudiment of the air-bladder has appeared as a small oval patch at the postero-dorsal aspect of the yolk-sac. Lumen appears in the embryonic gut. Fin fold around the tail has become broad. Chromatophores are more distinct than in the preceding stage. Embryo moves vigorously with the capsule, exerting pressure on its distal wall.

Embryo, 55 hours after fertilization: Stomodaeal depression is formed, sub-terminally ventral. Eyes are now glistening dark.

Embryo, 60 hours after fertilization: Owing to continued, vigourous movements of the embryo the egg capsule ruptures at the distal closed end and the embryo hatches out.

Hatching depends on the conditions of the environment, like temperature of water etc. All the eggs in the same brood do not

hatch out at the same time; in some the period of incubation being delayed by 4 to 8 hours. In certain cases it took about 72 hours for the embryos to hatch out.

(b) Larval Development

Hatchling : (Fig. 8). Extremely frail and almost transparent, the tiny hatchling measures about 2·4 mm. in length. Soon after hatching its movements are rather erratic; it first swims up quickly to the surface, then drops down quietly to the bottom where it lies quiet for a little while, only to repeat the same movements.

The head is conspicuous and blunt. In the dorsal view it is oval in shape, with the large eyes bulging conspicuously on either side. Mouth is open, but the lower jaw does not project beyond the upper. The yolk-sac is small and rounded; about 0·24 mm. in diameter, with a postero-ventral median groove and with the oil globule still persisting. Pectoral fins are flap-like. The air-bladder has become more conspicuous by the formation of pigment dorsally. Notochord is slightly arched at the level of the yolk sac. The anus is situated almost at the middle of the body. 26 myotomes could be counted. Pigmentation, but for the dark glistening eyes, is sparse. Scattered chromatophores are present on the yolk sac; while along the mid-ventral line, from the level of the anus backwards there are a few chromatophores.

Larva, 24 hours after hatching : It measures about 2·6 mm. in length. Lower jow is a little more pronounced than in the hatchling, with an acute projection near the base, ventrally. Pectoral fins are functional. Air-bladder has become dark and glistening. Yolk is largely absorbed, but the oil globule still persists.

Larva, 48 hours after hatching : (Fig. 9). Mouth has widened and the lower jaw has grown bigger. A very small portion of the yolk, together with the reduced oil globule remains to be absorbed. A more or less continuous row of chromatophores has appeared along the mid-ventral line. The larva measures about 2·7 to 2·85 mm. in length.

Larva, 72 to 80 hours after hatching : (Fig. 10). Yolk-sac, including the oil globule, is almost fully absorbed. Eyes and mouth have become very conspicuous, with the lower jaw slightly projecting. The larva about 3·1 to 3·3 mm. long, is still very slender and except for the pectorals, has no other fins differentiated. Air bladder has grown large. Chromatophores are more

prominent than in the previous stage, particularly near the air-bladder and the hind end of the gut.

After absorption of yolk it was found extremely difficult to rear the larvae in the laboratory aquaria. Artificial feeding with live plankton, egg yolk etc. was tried, but in no case the larvae survived after nine days. The stray specimens that lived for nine days did not show any further differentiation of structures than in the 3-4 days old larvae. A nine-day old specimen, still measuring only 3·3 mm. long, however, had begun feeding and was found to have taken in two rotifers and a diatom. Regular peristaltic movements of the gut could also be observed.

Discussion

The observation that *G. giuris* could be induced to deposit its eggs on objects specially provided for the purpose, is of considerable significance in carp culture. Because of its pronounced piscivorous tendencies, it is necessary to remove this species from carp nurseries and stocking ponds. In large pieces of water where dewatering is not possible and effective eradication of the species cannot be ensured by netting alone, a useful check on its population could possibly be maintained by inducing the breeders to deposit spawn on small planks and then removing the same from the pond before hatching.

Scattered notes on certain stages in the development of this Goby have been given by Willey (1911) and Raj (1916). The former found the egg tubes varying from 3 mm. to 8 mm. in length and the hatchling about 2·25 mm. long. He has also observed the reversal in the position of the embryo within the capsule and adds that just before hatching it is again found with the head pointing to the base of the egg tube. But for probably rare exceptions, this does not appear to be the normal condition and as already described, the head end of the embryo is almost invariably nearest the distal extremity of the capsule at the time of hatching. The above observation is in accord with those of Raj (loc. cit.), who has also figured some of the stages; the figures, however, being highly diagrammatic. Aiyar (1935) in his account of the development of *Acentrogobius neilli* does not mention this change in the orientation of the embryo as taking place in that species. From his figures also it cannot be clearly stated whether such a change takes place or not. In *A. viridipunctatus* also this re-orientation does not apparently take place (Jones, 1937). In *Boleophthalmus boddaerti* though Jones (loc. cit.) does not describe this change in position it is evi-

dent from his figures 58 and 59 that, as in *G. giuris*, the head end of the embryo which originally lies nearest the attached end of the capsule, comes to be directed towards the opposite end, as the embryo advances in differentiation.[3]

This change in the orientation of the embryo is probably intended to facilitate hatching. The head, differentiated at the anterior end of the yolk mass which contains the buoyant oil globules, is probably naturally directed upwards in the early stages, when the egg capsule is hanging down. The head thus directed towards the fixed end of the capsule will be less effective in enabling the embryo to rupture the capsule for hatching, than when it is directed towards the distal free end. Probably because of this, the embryo when it begins movement within the capsule, changes its position.

The protective egg capsule is a peculiar adaptation, common to several of the gobies. While in most cases, the capsule being short, the advanced embryo has to remain coiled within, till hatching, in *G. giuris* the elongated capsule enables the embryo to reach its full size without any coiling of the body. As the spawn is generally attached to the under surface of objects, the varying length of the egg capsules in one and the same brood might probably be an adaptation for presenting the maximum area of exposure in the limited space and thereby avoid possible sudden depletion of dissolved oxygen in the surrounding water. The parents, mounting guard on the spawn, might facilitate aeration by movements of fins.

Compared to the carps, the period of incubation, generally is much longer in the Gobies. In *G. giuris* it takes about 60 to 72 hours or even longer for the embryos to hatch out. The incubation period is even longer in *A. neilli* (Aiyar, loc. cit.) and *A. viridipunctatus* (Jones, loc. cit.). Compared to these smaller species (*A. neilli* and *A. viridipunctatus*) the *G. giuris* hatchling is very small and in certain respects like the absorption of yolk, development of caudal fin etc., much less advanced. The nature of the oil globules in the developing embryo of *G. giuris* is distinctly different from that in *A. viridipunctatus*. Even though at the time of hatching the larva is far better differentiated than in the carps, with the eyes fully developed, mouth well indicated, yolk more than half absorbed and the pectorals functional, it is still very small, very slender and less hardy than the carp hatchlings.

3. Chaudhuri (1951) also describes such a change in the position of the embryo in *Gobiopterus chuno*.

Summary

Glossogobius giuris breeds freely in fresh water ponds. It is a perennial spawner, with a peak period from September to December.

Breeding habits in fresh water ponds have been described, including a successful method for inducing the fish to deposit spawn on objects specially provided for the purpose. The possible significance of this method for effective control of the species in large nurseries and stocking ponds for carps, has been pointed out.

The early development has been followed in detail. A reorientation of the embryo within the egg capsule, taking place when it is fairly well differentiated, has been suggested as a possible adaptation on the part of the embryo to facilitate hatching.

The period of incubation, though much shorter than in some of the smaller gobies, is considerably longer than in carps. The embryos, as in other gobies, hatch out in a fairly well advanced stage but are decidedly small when compared to those of the smaller gobies *A. neilli* and *A. viridipunctatus.*

The yolk is completely absorbed by the third day after hatching, when the larvae probably begin feeding. Artificial feeding was not successful.

Acknowledgements

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REFERENCES

Aiyar, R. Gopala, 1935. Observations on the development of *Acentrogobius neilli* (*Gobius neilli, Day*). *Zool. Anz.,* Bd. 3.

Alikunhi, K. H. 1946. Annual Report for 1945-46; Madras Rural Piscicultural Scheme, I.C.A.R. Government Press, Madras.

Alikunhi, K. H. 1948. On the bionomics and post-larval development of the Orange-Chromide, *Etroplus maculatus* (*Bloch*), *Proc. 34th Indian Sci. Congr.,* New Delhi.

Bhattacharya, D. R. 1916. Stages in the life-history of *Gobius, Petrocirtes* and *Hemirhamphus. Mem. Indian Mus.,* Calcutta, Vol. V.

Guillermo, B.. and Villadolid, D. V. 1939. Fish fry industries of Philippines. *Philipp. J. Sci.* 69(1) ; 69-100.

Hora, S. L. 1936. Ecology and bionomics of the Gobioid fishes of the Gangetic delta. *Comptes Rendus Dn XIIe Congress International de Zoologic,* pp. 841-863.

Job, T. J. and Chacko, P. I. 1947. Rearing of salt water fish in fresh waters of Madras. *Indian Ecologist,* Vol. II, No. 1, pp. 1-9.

Job, T. J., *et al* 1948. On the biology of the Pearlspot *Etraplus suratensis* (Bloch). *Proc. 34th Indian Sci. Congr.,* New Delhi.

Jones, S. 1937. Observations on the breeding habits and development of certain brackish water fishes of Adyar, Madras. *Proc. Indian Acad. Sci.,* V, (6), pp. 261-89.

Koumans, F. P. 1941. Gobioid fishes of India. *Mem. Indian Mus.* XIII ; 205-329.

Mookerjee, H. K. *et al* 1947. On the food of *Glossogabiusgiuris* (Ham.) *Sci. and Culture*; XIII; 162-163.

Panikkar, N. K. and Aiyar, R. G. 1939. Observations on breeding in brackish water animals of Madras. *Proc. Indian Acad. Sci.,* IX, (6), pp. 343-364.

Raj, B. Sundara, 1916. Notes on the fresh water fishes of Madras. *Rec. Indian Mus.,* Calcutta, XII, pp. 249-294.

Willey, A. 1911. Notes on the fresh water fishes of Ceylon. *Spol. Zeyl.,* VII, pp. 88-105.

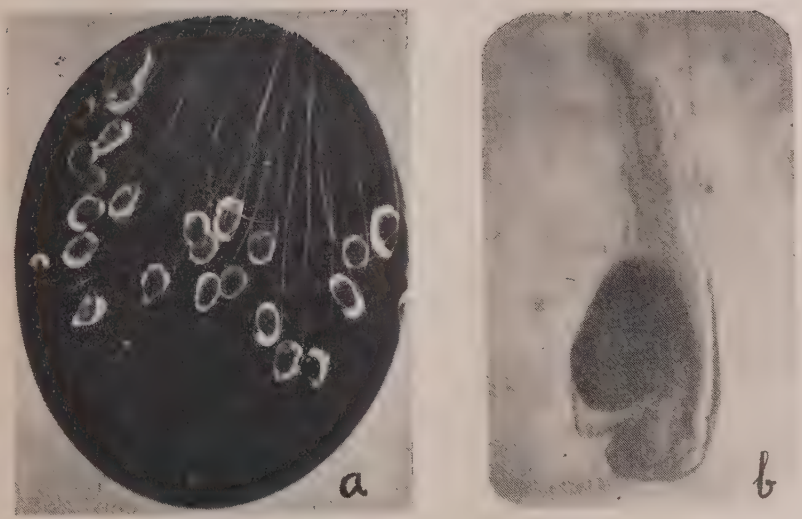
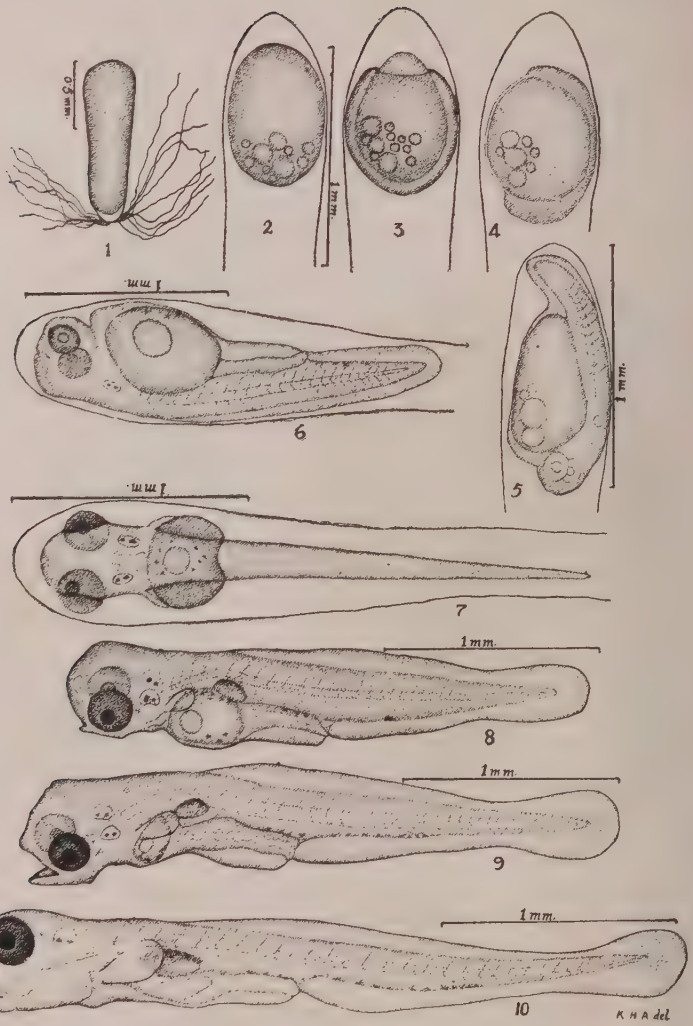


PLATE I

(a) Photomicrograph of a cluster of laid eggs of *Glossogobius giuris* (Ham.). Embryos in early stage with the head pointing towards the basal portion of egg tube, except in two specimens at the top and bottom extreme left in which the orientation has already changed. × 5.

(b) Magnified view of an embryo within the egg capsule, with head nearest the distal extremity. × 30.



EXPLANATION OF TEXT-FIGURES.

Glossogobius giuris (Hamilton), Fig. 1. ovarian egg, teased out. Fig. 2. fertilized egg, probably an hour after fertilization. Fig. 3. developing embryo, about 8 hours after fertilization. Fig. 4. developing embryo, about 10 hours after fertilization. Fig. 5. embryo, about 15 hours after fertilization. Fig. 6. advanced embryo, about 32 hours after fertilization. Note the position of head as compared to figure 5. Fig. 7 advanced embryo, about 48 hours after fertilization; dorsal view. Fig. 8. Hatchling, 2·4 mm. long. Fig. 9. Prolarva, 48 hours after hatching; 2·8 mm. long. Fig. 10. Prolarva, 76 hours after hatching; 3·1 mm. long.

SCAPOLITE CALC GRANULITE, PALLAVARAM

BY

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Abstract

An occurrence of scapolite bearing calc granulite and diopside granulite, is reported for the first time in association with the charnockites of the type area. A detailed microscopic description of the rock-types is given. From the characteristic mineral assemblages, it is concluded that the rocks are the products of high grade regional metamorphism of impure calcareous rocks. It is probable that the basic charnockites of the type area can also be regarded as derived from impure calcareous rocks as is thought to be the case in Bastar.

Introduction

An occurrence of calc-granulite with scapolite and diopside-granulite associated with garnetiferous leptynite and charnokite has been noted for the first time on a hillock, just to the east of the village of Old Pallavaram (Sheet $66^{D}/_{1}$ Lat. 12° 57½′; Long. 80° 11′), Chingleput District, (Madras). The occurrence of these rock types in Holland's[1] type area for charnockites may have some significance from the point of view of the origin of charnockites — a much debated problem.

Field Occurrence

The rock types described are sporadic and rare in occurrence and follow the general strike E.N.E. — W.S.W. with variations to E-W. The calc-granulites occur as thin bands and caught up patches in the diopside-granulite. They weather in a characteristic way, with knotty protuberances of comparatively unweathered portions of rocks. The weathering results in the rock mass as a whole appearing somewhat greyish dark.

Description of the Rock Types

Scapolite calc-granulite :

The rock is greyish white with coarse calcite, dark green grains of pyroxene and honey-coloured scapolite. The texture is coarse and porous. Occasionally ptygmatic folding and crinkling of the bands could be seen in hand specimens.

Under the microscope the minerals observed are :—scapolite, calcite, plagioclase, garnet, green pyroxene, quartz, sphene and apatite. The silcates are typically anhydrous, as those found in some igneous rocks. Hydrous silicates like amphiboles and micas are absent.

The texture is characteristically granulitic (Pl. I, Fig. 1), with some variation in the size of the mineral grains, due to differential growths in the solid medium.

Minerals

Scapolite :

The mineral does not show idiomorphic outlines. Basal sections reveal two cleavages at right angles to each other. The parallel extinction, negative uniaxial character, the strong double refraction giving interference colours of the second order, are diagnostic.

The measured optical values are :

(1) Indices of Refraction :

N_o	..	1·603
N_e	..	1·564
Birefringence, $N_o - N_e$	..	0·039
(2) „ „	..	0·038 (by Ehringhaus Compensator).

The Indices of Refraction were determined by liquids in basal and prismatic sections. The R. I. of liquids were simultaneously determined at room temperature (32° C.) by Abbe Refractometer, with sodium vapour lamp.

The high value of birefringence 0·038. according to Winchell,[2] corresponds to the chemical composition of :—

Marialite ($Na_3AlSi_3O_8$ NaCl)	..	18%
Meionite ($Ca_3Al_6Si_6O_{24}CaCo_3$)	..	82%

The scapolite is mostly clouded in the centre (Pl. I, Fig. 2). It looks as though the mineral is moulded between felspar pieces ; the felspars are really relics (Pl. I, Fig. 3) which have escaped scapolitization as clearly seen by the inverse relationship of the two minerals in their abundance.

Calcite :

It is recognised by its very high birefringence, perfect cleavages and negative uniaxial character. Its assocation with quartz is significant.

Plagioclase :

The plagioclases are twinned on the albite and pericline laws : the twinning lamellae are often bent and the extinction wavy.

The extinction on (010) from (001) cleavage on selected pieces gives an average of 32°. 2V is 84° (—)ve, as determined by universal stage. The optical data indicate Ab_{25} An_{75} as the composition of the plagioclase.

Pyroxene :

The pyroxene in the rock occurs as dark green grains with cracks. (110) cleavage and occasionally polysynthetic twinning lamellae are observed. 2 V is 62° with Z as acute bisectrix as measured by universal stage.

The two determined Indices of Refraction are :—

N_p		..	1·727
N_g		..	1·747
Birefringence :	$N_g - N_p$	=	0·020
C $\wedge$ Z		..	42°

The optical values show a composition of Di_{20} He_{80} as read from Winchell's[2] graphs. Rarely the pyroxene is found altering to hornblende.

Garnet :

The mineral is flesh-coloured, isotropic with a number of cracks.

The Index of Refraction is : 1·739 ± ·001.

(The R.I. of the liquid was determined simultaneously at room temperature by Leitz-Jelly Micro-Refractometer).

The composition, according to Fleischer[3] is :—

Grossularite	..	96·75%
Andradite	..	2·27%
Almandine	..	0·59%
Uvarovite	..	0·39%

Inclusions of pyroxene and plagioclase are found in the garnet (Pl. I, Fig. 4).

Sphene :

This is fairly abundant and pleochroic from colourless to pink. *Quartz* and *apatite* are also present.

Diopside-granulite :

Megascopically the rock is medium-grained and greyish black; in hand specimens the rock is easily mistaken for a norite. The minerals observed under the microscope are: plagioclase, pyroxene, garnet, quartz, sphene and apatite. Scapolite and calcite are absent. There is every gradation from the scapolite calc-granulite to diopside-granulite.

Discussion

The mineral assemblages and the measured optical values clearly indicate that the rocks are rich in CaO and SiO_2. FeO, MgO, Al_2O_3 and TiO_2 are present, the last two in small amounts. The mineral assemblages can be represented by a tetrahedron (Text Fig. 1) with one component at each apex. The tetrahedral faces will constitute the four ternary systems CaO MgO SiO_2, CaO Al_2O_3 SiO_2, MgO Al_2O_3 SiO_2 and a front face CaO MgO Al_2O_3.

If the front face is removed, it will expose the interior of the tetrahedron with the minerals calcite, diopside, anorthite and quartz.

In the tetrahedron whose apices are represented by these four minerals, we do not have the plane, wollastanite, anorthite and diopside, which would have made the presence of calcite and quartz incompatible with each other.

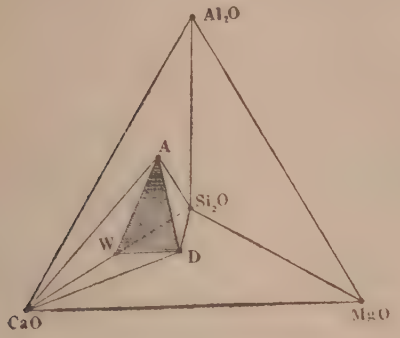

FIG. 1
Diagram representing the quarternary system $CaO - MgO - Al_2O_3 - SiO_2$, as a tetrahedron with one component at each apex.

In the A.C.F. diagram the mineral assemblages occupy the fields 1 & 2.

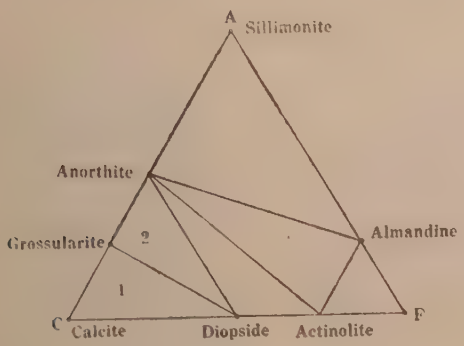

FIG. 2
A.C.F. diagram for rocks, with excess $Si\ O_2$.

The mineral assemblages of the two rock-types suggest that they are the products of high-grade regional metamorphism of impure calcareous rocks.

The presence together of calcite and quartz in the scapolite calc-granulite suggests that the prevailing pressures should have been high enough to inhibit the formation of wollastonite. However, the rock cannot be classified under the granulite facies (plutonic metamorphism without stress) as sphene which is invariably absent in the granulite facies[4] is fairly abundant in the rock. So it has been assigned to the sillimanite zone of regional metamorphism, where also the pressures are too high to allow the

formation of wollastonite. Further, as Harker[5] says, the relatively yielding nature of a rock composed of calcite makes "any very high measure of shearing stress impossible."

The diopside-granulite is also to be taken as belonging to the same facies, the mineralogical difference (absence of free calcite) being of course attributable to the original difference in composition.

It is suggested that the scapolitization of the plagioclase has taken place in the process of regional metamorphism of the calcareous rocks. The necessary volatile constituents have presumably been derived from the material already present in the rocks.[4] This receives support from the presence of 82% of meionite molecule in the scapolite. Had the scapolization been due to the introduction of the volatiles Na & Cl, it should have been richer in the marialite molecule.

The study of the rock types closely associated with the charnokites in the type area makes one doubt the igneous theory so ably advocated by Holland for the origin of charnockites.

It is obvious that the scapolite calc-granulite and diopside-granulite have resulted from impure calcareous rocks by high-grade metamorphism. The problem is to consider whether the charnockites are the variants of the calc and diopside granulites. With regard to the charnockites of Bastar, we are aware that Ghosh[6] has shown that they are metamorphosed impure calcareous rocks (diopside gneisses) which have been progressively granitized. Indeed, while Ghosh has not met with free calcite in his rocks, it is very interesting that the diopside granulite in the type area should show a clear gradation to actual calc-granulite.

Ghosh's view is increase in the grade of metamorphism has resulted in the development of (a) hypersthene, secondarily from diopside; (b) and of garnet from diopside and plagioclase.

Microscopic examination of charnockites of Pallavaram lends support to the views of Ghosh.

(1) Diopside is found changing to hypersthene. This is obvious from the fact that diopside grains that surround the hypersthene are in optical continuity.

(2) Grains of diopside and plagioclase are found in garnet.

Fig. 1

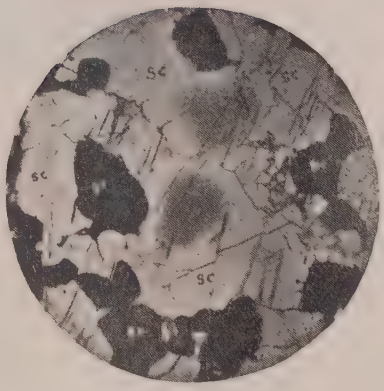

Fig. 2

Fig. 3

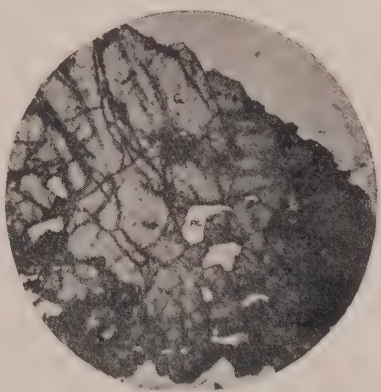

Fig. 4

Fig. 1. Scapolite (Sc); diopside (Di) and apatite (Ap) in scapolite calc granulite × 14. Fig. 2. Scapolite (Sc); diopside (Di); scapolite calc granulite × 14. Fig. 3. Scapolite (Sc); Plagioclase (Pl); Diopside (Di); scapolite calc granulite × 60. Fig 4. Garnet (G); Plagioclase (Pl) and pyroxene (Py); diopside (Di); diopside granulite × 25.

It is therefore suggested that the basic charnockites could have resulted from the granulites here described, by an increase in the grade of metamorphism.

It is clear that excepting for local occurrences of the calc-granulite and diopside-granulites which have been referred to the sillimanite zone of regional metamorphism, the general metamorphic impress has been still higher—of the granulite facies.

Ghosh derives the intermediate and acid members of the charnockites from the basic types, by influx of alkalies. Detailed studies of the charnockites of the type area are in progress to find out whether the interesting reaction phenomena noted by Ghosh, are present here also. The observations will form the subject of a separate contribution.

REFERENCES

1. Holland, T. H. "The Charnockite Series . . . . " *Mem. Geol. Surv. Ind.*, XXVIII, Pt. 2, 1900.

2. Winchell, A. N. "*Elements of Optical mineralogy*" Part 2.

3. Fleischer, M. "Relation between chemical composition and the physical properties in the Garnet Group." *Am. Min.* Vol. 22, 1937, pp. 751-759.

4. Turner, F. "The Mineralogical and Structural Evolution of Metamorphic Rocks." *Geol. Soc. America Mem.* 30.

5. Harker, A. "*Metamorphism*", 1939.

6. Ghosh, P. K. "The Charnockite Series of Bastar State . . . . " *Rec. Geol. Surv. India*, LXXV, Paper 15, 1941.

DEVELOPMENT OF A HARPACTICOID COPEPOD, *MACROSETELLA GRACILIS* (DANA)

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INTRODUCTION

Macrosetella gracilis (Dana) is a tropical form which appears to be widely distributed. Though the external morphology of this Harpaticoid has been described by Dana (1873), Brady (1883), Giesbrechet (1892), and Wilson (1932) yet the development has not been studied. Large numbers of larvae of this form occur in September and October and it was possible to collect about 300 of these in different stages from the inshore plankton. From this material 6 naupliar and 6 copepodite stages were obtained. Some ovigerous females were reared to get the first nauplius.

DEVELOPMENTAL STAGES

Eggs: The eggs are coloured light yellow and are carried in external brood-pouches in two bunches of 7-8 each. The diameter of the egg varies from 0·06 to 0·08 mm.

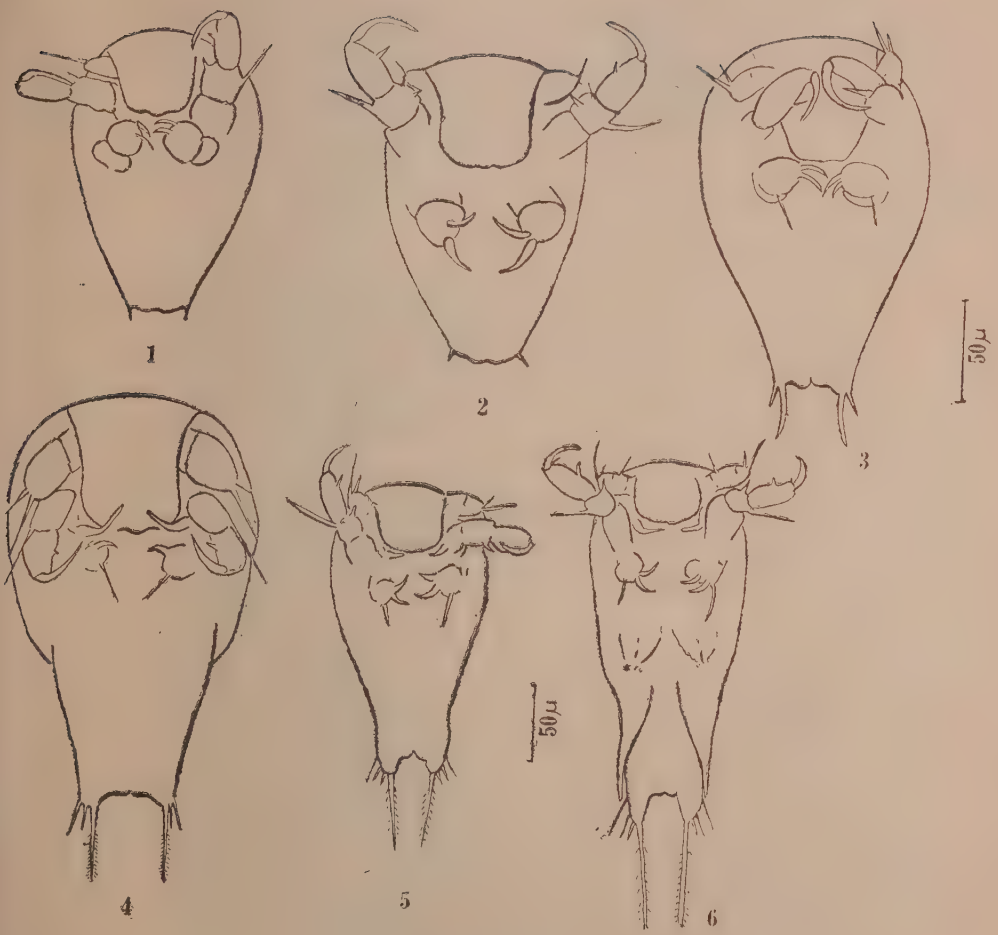
Naupliar Stages:

Though the typical Harpacticid nauplii are said to be creepers on the bottom, the present forms, through their association with the planktonic alga *Trichodescium* live at the surface and show several noteworthy features. The poor development of the *antennule*, the well developed antenna facilitating prehension, are peculiar to this form.

Stage I (Fig. 1): Length — 0·102 mm. The nauplius is more or less triangular in shape. Red pigments are present inside the body. The eye appears as a red spot. Labrum is prominent. There are two short posterior spines. (Fig. 7).

Antennule: Rudimentary with one long apical seta (Fig. 13).

Antenna: Well developed, two jointed, the terminal joint being armed with a claw. The inner margin of the first joint is armed



Text Figs. 1 to 6.

Figs. 1 to 6 Naupliar stages 1 to 6.

with a spine and the end of the outer margin is drawn out into a spinous process (Fig. 19).

Mandible: Single lobed, armed with two curved setae. (Fig. 25).

Stage II (Figs. 2, 8, 14, 20 and 26)

Length 0·129 mm. posterior spines more robust than in Stage I. There is no appreciable change in the number or structure of the appendages.

Stage III (Fig. 3).

Length 0·169 mm. The nauplius is pyriform and is broader than long. The posterior region carries a pair of spines pointing downwards in addition to a pair of setae and a pair of bristles (Fig. 9).

Antennule: single-jointed armed with apical setae (Fig. 15).

Antenna: as in Stage I (Fig. 21).

Mandible: Indistinctly bilobed with two curved spines and a long seta which points downwards (Fig. 27).

Stage IV (Fig. 4).

Length 0·195 mm. Pyriform, longer than broad. The posterior ends with two pairs of long setae which are armed with spinules and two pairs of naked setae (Fig. 10).

Antennule: as in previous stage. (Fig. 16).

Antenna: as in previous stage with the addition of a spinous process at the base of the claw (Fig. 22).

Mandible: more prominent than in Stage II, with an increase in the length of one of the spines (Fig. 28).

Stage V. (Fig. 5).

Length 0·33 mm. The naupulius is narrower than long. The dimension of the larva of this stage shows a sudden increase on the previous stage. The posterior spines show an increase in length and one more pair of setae are added to the existing numbers (Fig. 11).

Antennule: Indistinctly two jointed, the terminal joint with two setae as in Stage IV. (Fig 17) *Mandible* as in previous forms

but shorter in length. (Fig. 29) and *Antenna* as in Stage IV (Fig. 23). *Maxillae* rudimentary.

Stage VI (Fig. 6).

Length 0·815 mm. The body is nearly 2½ times longer than broad. Posterior spines have increased in length (Fig. 30). Traces of first and second swimming feet are present.

Antennule : Two jointed, first joint with a seta on its inner side and the terminal joint with two apical setae (Fig. 18).

Antenna as in Stage V. One more teeth like projection is added to the first joint of the Antenna (Fig. 24). *Mandible* as in stage V (Fig. 30) *Maxillae* rudimentary.

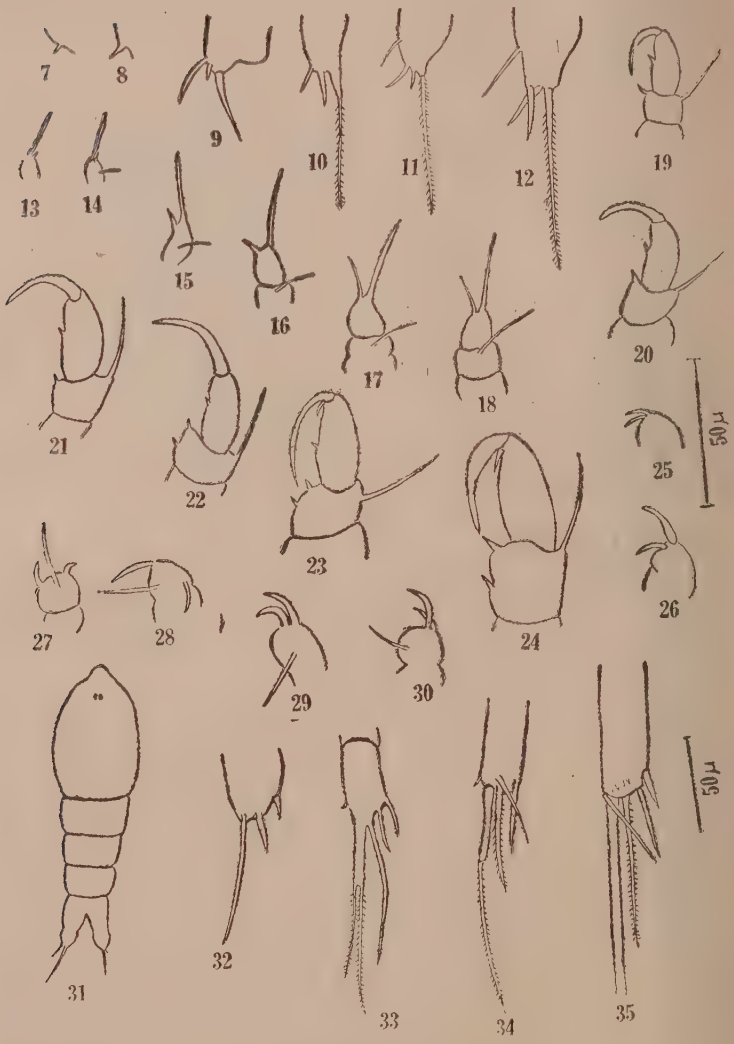
Copepodites : There are six copepodite stages as in all other copepods, the sixth being that of the mature adult. Sexes are distinguishable from the fourth stage onwards.

Copepodite I (Fig. 31).

Length 0·484 mm. The body is composed of five segments including the *Cephalosome.* Eye is distinct. There is a rapid increase in size. Three pairs of swimming feet are present, the third pair being rudimentary.

Antennule : Five jointed. The outer margin of the first joint is produced into a spinous process. The second joint bears two setae, third, one seta, and, the fifth two apical setae (Fig. 36). *Antenna* : two jointed with three apical setae, the middle one being the longest (Fig. 41). *Mandible*—simple, with a pointed tooth; palp absent. *First Maxilla*—rudimentary, represented by a broad triangular plate with undulated margin *Second Maxilla*—Indistinctly bi-lobed, the outer lobe carrying three spines (Fig. 46). *Maxilliped.*—Two jointed. (The first joint loses the teeth which were prominent in the naupliar stages.) The terminal joint bears four small, pointed spinules and an apical curved claw with a small teeth-like projection at its base. (Fig. 50). *First swimming feet*—biramous with an unjointed endopodite and exopodite. The endopodite carries two apical and one sub-apical seta and the exopodite bears two apical and two short sub-apical seta, one of which is found about the middle of the outer margin (Fig. 53).

Second swimming feet indistinctly biramous, the outer lobe with a spine and two long apical setae and the inner lobe with two setae (Fig. 59).



Text Figs. 7 to 35.

Figs. 7 to 12	Posterior region of Nauplii 1 to 6.
Figs. 13 to 18	Antennule in Naupliar stages.
Figs. 19 to 24	Antenna "
Figs. 25 to 30	Mandible "
Figs. 31	Copepodite-Stage I.
Figs. 32 to 35	Caudal ramus of Copepodite stages.

Third swimming feet—rudimentary. *Armature of the caudal furca.* The line of division between the abdominal joint and furca is indistinct. Each ramus carries a plumose spine towards the inner side and two setae towards the outer side (Fig. 32).

Copepodite II.

Length 0·616 mm. The body is composed of five segments. The spines in the caudal furca show an increase in length. There are three pairs of swimming feet, the third being rudimentary.

Antennule : Five jointed, second joint shows an increase in size. The outer marginal ends of the first and fourth joints are produced into spinous processes. The third joint bears three setae and the terminal joint three short sub-apical and two long apical setae (Fig. 37).

Antenna: Two jointed and traces of a third joint are present (Fig. 42) armature as in Stage I. There is no appreciable change in *Mandible, Maxillae* and *Maxilliped.*

First swimming feet : The basipodite is produced into a spinous process towards the outer marginal end. The single jointed exopodite as well as the endopodite are armed with a spine each towards the outer and inner margins respectively. The exopodite carries a small spine and two setae while the endopodite bears a spine and two setae (Fig. 54).

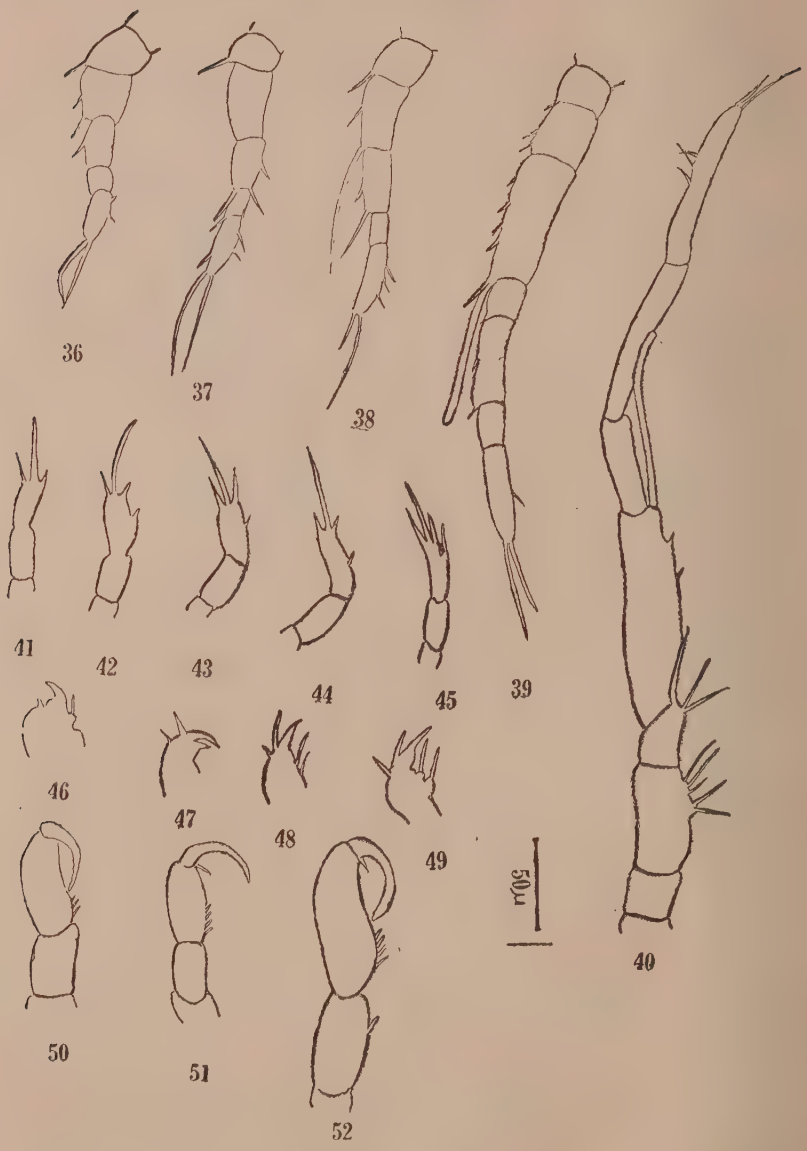
Second swimming feet : Biramous, the exopodite as well as the endopodite is constricted about the middle of the joint, indicating future segmentation. The exopodite bears two lateral spines and two apical setae. The endopodite is shorter than the exopodite and is armed with two apical setae and a lateral spine at the inner margin (Fig. 60).

Third swimming feet : Rudimentary, indistinctly bilobed the outer lobe with one spine and two setae, the inner lobe with two setae (Fig. 64).

Armature of the furcal ramus : The innermost spine shows an increase in length. There are two short sub-apical setae, a long apical seta and a bristle above the base of the innermost spine (Fig. 33).

Copepodite III

Length 0·742 mm. There are seven segments in the body including the cephalosome. Four pairs of swimming feet are present. There is no change in the mouth parts.



Text Figs. 36 to 52.

Figs. 36 to 40 Antennule in Copepodite stages.
Figs. 41 to 45 Antenna.
Figs. 46 to 49 Maxilla 2.
Figs. 50 to 52 Maxilliped.

Antennule : Five jointed, the first joint as in Stage II. The second joint bears a spine and an aesthetask towards the outer margin. The third joint bears two setae and the terminal joint three short lateral spines about the middle of the outer margin and two apical setae (Fig. 38).

Antenna : Three jointed, the terminal joint with three apical and one sub-apical seta (Fig. 43). *Mandible, Maxillae* and *Maxilliped* (Fig. 51) as in Stage II.

First swimming feet: Basipodite as in Stage II. The endopodite as well as the exopodite is two jointed. The outer marginal ends of the exopodite as well as the inner marginal ends of the endopodite joints are produced into spinous processes. The terminal exopodite joint bears three apical setae and that of the endopodite two short apical setae with a long spine in between (Fig. 55).

Second swimming feet : The exopodite is two jointed, the outer distal marginal end of the first joint is produced into a spine, the second joint carries two apical spines and three setae. The endopodite shows a constriction about the middle of the joint and carries two apical setae and two short spines (Fig. 61).

Third swimming feet : Not well developed. It is biramous, the outer lobe with a spine and two setae and the inner lobe with two setae (Fig. 65).

Fourth swimming feet : Rudimentary indistinctly bilobed, the outer lobe with one spine and two setae and the inner lobe with two setae (Fig. 68).

Armature of the furcal ramus : There are two small bristles, one towards the outer margin and one towards the inner margin respectively. There are two apical plumose spines and two subapical setae. There is a seta starting from the base of the plumose spines (Fig. 34).

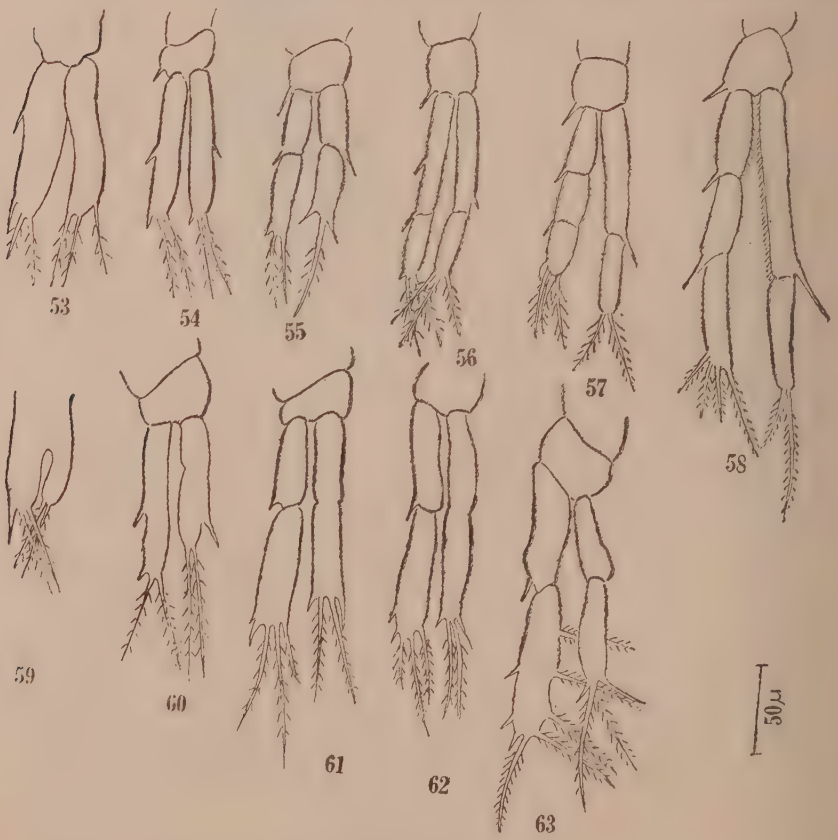
Copepodite IV

Length 0·92 mm. Body is seven segmented. Sexes are distinguishable. Rudimentary fifth legs are present.

Antennule : Seven jointed, first joint as in previous stage. The second joint carries two short spines, third joint four short lateral spines and an 'aesthetask', fourth joint one short spine, fifth two short lateral spines and the seventh two apical setae and a short spine about the middle of the inner margin (Fig. 39).

Antenna : (Fig. 44). *Mandible, Maxillae* and *Maxilliped* as in Stage III.

First swimming feet : Basipodite as in Stage III. The endopodite as well as the exopodite shows an increase in length. The first joint of the exopodite is armed with two spines, one towards the middle and one at the distal end of the outer margin. The second joint is nearly half as long as the first and carries one apical and two sub-apical setae. The endopodite is also two jointed and



Text Figs. 53 to 63.

Figs. 53 to 58 First swimming feet.
Figs. 59 to 63 Second swimming feet.

is as long as the exopodite. The inner margin of the first joint bears one short spine while the second joint carries three setae at the apex (Fig. 56).

Second swimming feet : Exopodite two jointed and the armature as in Stage III. Endopodite with two spines and two setae (Fig. 62).

Third swimming feet : The exopodite as well as the endopodite shows a constriction about the middle of the joint. The exopodite is armed with a short spine about the middle of the outer margin and one apical and four sub-apical setae towards the inner margin of the distal end. The endopodite carries one apical spine and two apical setae and two sub-apical lateral setae (Fig. 66).

Fourth swimming feet : Biramous with an unjointed exopodite and endopodite. The exopodite bears three setae and two spines and the endopodite bears three setae (Fig. 70).

Fifth feet: Rudimentary. Male: two jointed. The inner margins of basal expansion produced into two spinous processes. The distal joint bears two spines and one seta with enlarged base (Fig. 74).

Female : The basal expansion tipped with two setae towards the inner side and one towards the outer side. Distal segment with one sub-apical spine towards the outer margin, two short apical setae and a spine in between. (Fig. 72).

Copepodite Stage V.

Sexes are distinguishable clearly at this stage. The male differs from the female not only in size but also in the form of the Antennule and of the fifth leg.

The female : Size Length 1·27 mm.

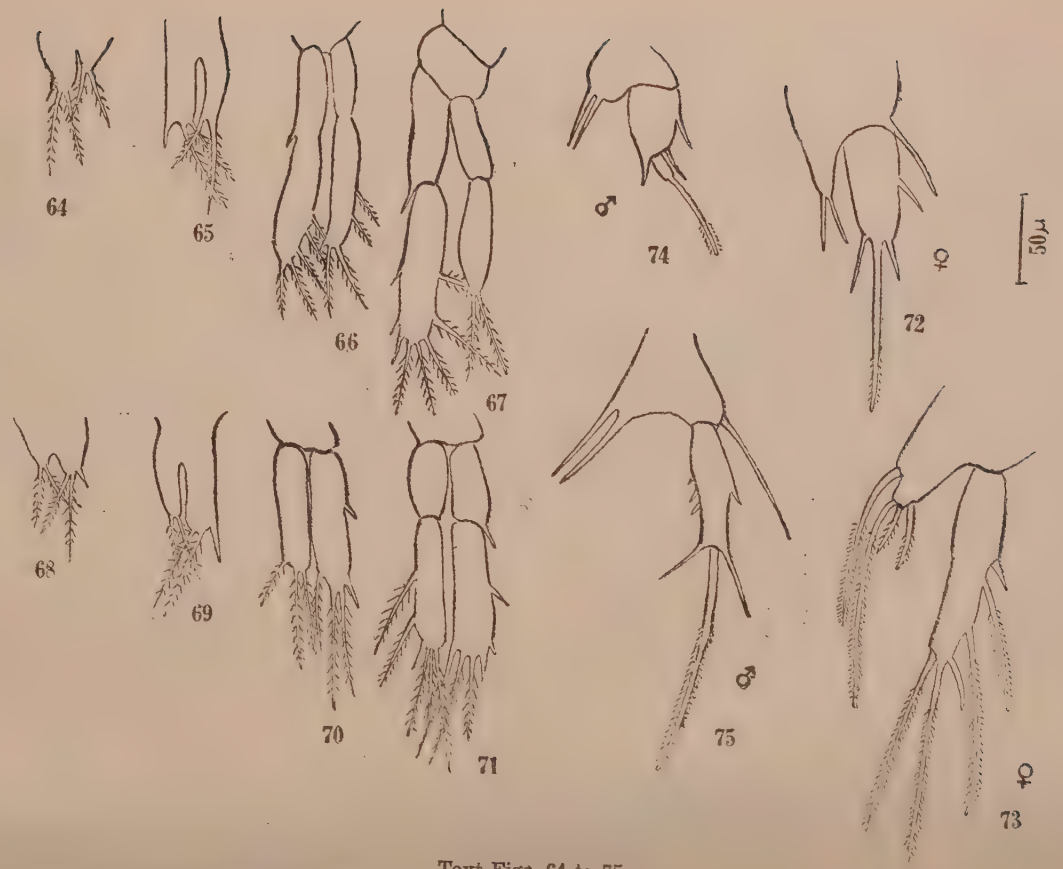
Antennule : Eight jointed sparingly setose. The first, second and third joints bear a seta each towards the outer margin. The fourth joint bears an aesthetask. The terminal joint carries a seta.

Antenna : Three jointed, terminal joint with three apical and one sub-apical seta (Fig. 45).

Mandible : Rudimentary consisting of a cutting part and a seta representing the palp. *First Maxilla* rudimentary with an irregular free-edge. *Second Maxilla* three lobed. First lobe carries three spines the one in the middle being very stout, the second and third lobes carry one spine each (Fig. 49).

Maxilliped: Two jointed. The first joint bears three short bristles towards the inner margin. The second joint bears seven setae and an apical claw (Fig. 52).

First swimming feet : Basipodite as in other stages. The endopodis longer than the exopod. There is a short spine at the inner marginal end of the first joint of the endopodite and the second joint bears two setae at the apex. The exopodite is three jointed,



Text Figs. 64 to 75.

Figs. 64 to 67 Third Swimming feet.
Figs. 68 to 71 Fourth " "

Figs. 72 to 73 Fifth leg—Female.
Figs. 74 to 75 Fifth leg—Male.

TABLE I

Nauplii

Stage	I	II	III	IV	V	VI
Length in mm. (with posterior setae)	0·102	0·130	0·168	0·205	0·302	0·369
Length in mm. (without setae)	0·100	0·128	0·163	0·193	0·234	0·285
Antennule	1 jointed 1 seta	1 jointed 3 setae	1 jointed 3 setae	2 jointed 3 setae	2 jointed 3 setae	2 jointed 3 setae
Mandible	2 spines	2 spines	2 spines 1 seta	2 spines 1 seta	2 spines 1 seta	2 spines 1 seta
Antenna	2 jointed	2 jointed	2 jointed	2 jointed	2 jointed	2 jointed
Posterior region	2 short bristles	2 short bristles	4 short setae 2 bristles	2 plumose spines 2 setae	2 plumose spines 6 setae	2 plumose spines 8 setae

TABLE II

Copepodites

Stage	I	II	III	IV	V	VI
Length in mm. (with caudal seta)	0·484	0·616	0·742	0·922	1·27	1·4
Length in mm. (without caudal seta)	0·348	0·410	0·452	0·874	0·750	0·983
No. of joints in the metasome	4	4	4	4	5	5
No. of joints in the urosome	1	2	3	3	4	4
(No. of joints)	5	5	5	7	7 8	4 7
Antenna			A* present	A* present	A* present	8
(No. of joints)	2	2	3	3	3	3
Swimming feet	2 1 R	3 1 R	3 1 R	4 1 R	5	5
Caudal ramus	3 setae	4 setae 1 bristle	4 setae 1 bristle	2 setae 1 bristle	2 setae 1 bristle	2 setae 1 bristle

* A = Aesthete

the outer distal ends of first and second joints are produced into spinous processes, and the third joint bears four setae (Fig. 57).

Second swimming feet: The endopodite as well as the exopodite is two segmented. The second endopodite joint bears a spine and two setae. The first exopod joint bears a spine, the second, three spines and five setae (Fig. 63).

Third swimming feet: Exopod as well as endopod two jointed. The terminal endopodite joint bears one spine and two setae and the first exopodite joint carries a spine, the second three spines and 5 setae (Fig. 67).

Fourth swimming feet: With two jointed exopod and endopod. The terminal joint of the endopodite is armed with a spine and three setae. The first joint of the exopodite bears one spine and the second joint three spines and three setae (Fig. 71).

Fifth leg: Two segmented. The basal expansion with two short and two long plumose setae towards the inner side. The distal segment is armed with one sub-apical and one lateral spines and two sub-apical setae towards the outer side and two plumose setae at the apex (Fig. 73).

The Male: Length 1·1 mm.

Antennule: Seven jointed. Geniculate; hinged between the fifth and sixth joint. The second joint bears five setae, third three setae, fourth two spines and an aesthetask and the seventh three lateral spines and two apical setae (Fig. 40).

Antenna, Mandible, Maxillae and *Maxilliped* as in female as also the first four pairs of swimming feet.

Fifth feet: The basal expansion with two inner setae and one outer setae. The distal joint is long, and its posterior ends are produced into acute processes. There is a seta in between. There are 3 small teeth on the inner side (Fig. 75).

Sixth copepodite or the adult: The description is omitted as it has been figured and described by Brady (1873, Pl. L), Giesbrecht (1893 Pl. 45) and Wilson (1932, p. 282, Fig. 174).

Diagnostic features of the Nauplii and Copepodites

From the description of the larvae and the appendages given above it will be seen that the various stages of the nauplii and copepodites possess several features by which they can be identified. This can be summarised and tabulated for easy reference as follows: (Tables I & II).

Remarks:

As will be seen from Tables I and II the oldest nauplius measures 0·369 mm. when this moults, the earliest copepodite emerges as a form 0·484 mm. long. This sudden increase in size in the first copepodite is noteworthy. Similar sudden increase was found in *Euchaeta* by Nicholls (1933). This may be due to the first copepodite being equipped with a larger compliment of natatory appendages necessary for a free-swimming life but absent in the naupliar stages. The lack of masticatory spines in the oral appendages, and the presence of yolk in large quantities shows that the nauplii do not feed as in *Misophria* (Gurney 1930) and *Euchaeta* (Nicholls loc. cit.). The nauplius is also symmetrical as in other Harpacticoids.

Summary

1. The eggs as well as the six naupliar and six copepodite stages of *Macrosetella gracilis* are described.

2. The gradual differentiation of the structure of the appendages in the copepodite stages, is traced.

3. The distinctive features of each of the naupliar and copepodite stages are briefly enumerated.

Acknowledgements

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REFERENCES

Brady, G. S. 1883, Copepoda. Challenger Reports. Vol. VIII, pp. 108-109.

Brian, A. (1921) I. "Copepodi Harpacticoidi del Golfo de Genova" *Stud. Lab. Mar.* Genova.

Fraser, J. H. 1935-36. The occurrence, ecology and life history of *Tigriopus fulvus, J. Mar. biol. Ass. U.K.* (N.S.) Vol.,XX, pp. 523-33.

Giesbrecht, W. 1892. *Fauna Und Flora des Golfes von Neapel,* Vol. 19.

Gurney, R. 1931-32. British Fresh Water Copepoda. 3 Vols. Ray Society.

——1933. Notes on some Copepoda from Plymouth. *J. Mar. biol. Ass. U.K.* (N.S.) Vol. *XIX,* pp. 299-304.

Johnson, M. W. 1934. The Life history of the copepod *Tortanus discaudatus. Biol. Bull. Wood's Hole,* Vol. 67.

Johnson, M. W. and Obson, J. B. 1948. The Life history and biology of a marine Harpacticoid, *Tisbe furcata* (Baird). *Biol. Bull. Wood's Hole*, Vol. *95*, p. 320.

Krishnaswamy, S. 1948. Stages in the development of *Harpacticoid copepod, Macrosetella gracilis* (Dana). *Curr. Sci.* Vol. 17.

—— 1950. Breeding seasons of the copepods in the Madras Plankton. *Proc. Indian Sci. Congress.* 37th Session.

—— 1950. Larval stages of some copepods in the Madras Plankton and their seasonal fluctuations. *J. Madras Univ.* B. Vol. *XIX.*

Nicholls, A. G. 1933-34. The developmental stages of *Euchaeta norwegica* Boeck. *Proc. Roy. Soc. Edin. Vol.* LIV, pp. 31-50.

—— 1941. The developmental stages of *Metis joussemei* Richard, *Ann. Mag. nat. Hist.* Vol. *VII*, pp. 317-328.

Wilson, C. B. 1938. Copepods of the Wood's Hole Region Massachusetts, *Bull U.S. nat. Mus.* No. 158.

FATTY ACIDS AND GLYCERIDE COMPOSITION OF SESAMUM INDICUM

BY

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Sesame oil also known as benne, till, gingelly and many other names is obtained from the seeds of a herbaceous plant, *Sesamum indicum*, of which several varieties and sub-species are known. Sesame belongs to the family of Pedaliaceae which is sometimes annexed to the Bignoniaceae. Since the sesame plant has been cultivated from time immemorial the original home is unknown. According to A. de Candolle[1] sesame seed was brought from the Sunda islands to India several thousands of years ago and has migrated thence through the Euphrates basin to Egypt. Marco Polo mentions the manufacture of sesame oil by expression of the seed in Abyssinia, Ceylon and the Malabar Coast.

Sesame plants usually grow from two to four feet high, sometimes much higher also. The shape of lower leaves are generally broad, and coarsely toothed or lobed while the upper leaves are lanceolate. The flowers which vary in colour from pinkish to yellow are tubular and two lipped and about three quarters of an inch in length. They have four stamens of unequal length. The seed pod is two valved and contains numerous small seeds. When matured the pods open and much of the seeds are often scattered around.

Linnaeus differentiated two species, viz., *Sesamum indicum*, producing white or yellow seeds and *Sesamum orientale*, L, which produces dark (red, brown or black) seeds. Commercially the seed is known as white and black. The white seeds yield oil superior in quality to that from the dark coloured variety. Technically the white seed is termed *Suffet-til* whilst the black variety containing the largest proportion of oil bears the name, *bigarre*.

In addition to the use for the production of oil in various countries the seed is used to some extent in making various kinds of confectionery. Both seed and leaves are used as demulcents and for other medical purposes.

Sesame oil is much used as a salad and cooking oil as well as in the manufacture of shortenings (lard substitutes). The oil is also used in pharmacy, soap making, as a burning oil and for making India-rubber substitutes besides for other minor purposes.

Utz[2] has tested African, Indian and Levantine sesame oils and his analysis was restricted to the specific gravity, melting point of fatty acids, polarisation in a 200 mm. tube, iodine number, refraction at 25°C and 40°C and refraction of fatty acids at 25°C and 40°C. Sprinkmeyer and Wagner[3] confirm that the African oil has the highest rotation whereas they find the lowest rotations in Indian oils.

Lane[4] made a more detailed examination by the lead-salt-ether method. He obtained 78·1% of liquid fatty acids by this method. These consist of oleic and linoleic acids. He obtained by means of the linoleic tetrabromide estimation, 16·4, 15·2 and 12·6% of linoleic acid. From the composition of 12·19% solid acids, 15·8% linoleic and 72·1% oleic acid the iodine value of the mixed fatty acids would come to 93·6 whereas the direct determination gave the mean value of 111. Hence his figures can be accepted only with reserve. The iodine values of 37 samples of sesame oil pressed from seeds of various origins were found by Wijs[5] to range from 106·1 to 116·8, the oils from the second pressings gave values ranging from 105·2 to 110·3 and the third pressings from 103·9 to 109·8.

Sprinkmeyer and Wagner[6] have made a comparative study of physical properties of Indian, Levantine and African seed oils. The values are given in the following table.

Their analysis showed that there is little difference between the cold and hot pressed oil. The African oil yielded constants which differed considerably from those given by the other two varieties, for the iodine value of African oil was 114 whereas the other specimens had less iodine value. This was probably due to the change of climate of the countries in which the seed was habitated.

Hefter[7] has reported merely the constants of the oil.

More recently, Jamieson and Baughman[8] investigated in detail a specimen of sesame oil. The oil was expressed by means of an expeller from pale yellow seeds grown in China. The seed con-

CONSTANTS OF AFRICAN, INDIAN AND LEVANTINE SESAME OILS

	African		Indian		Levantine	
	W.	S. & W.	W.	S. & W.	W.	S. & W.
Sp. gravity at 15·5°C. ..	0·9232		0·9218		0·9220	
Rotation 15°C. ..	+1·6°	+1·42°	+1·4°	+1·03	+0·8°	+1·11°
Butyro refractometer degrees at 25°C ..	67·5	69·2	66·2	68·2	67·0	68·0
Butyro refractometer degrees at 40°C. ..	59·5		58·2		59·1	
Iodine value ..	106·3	114·11	104·8	108·31	107·7	108·84
M. P. of mixed fatty acids, °C. ..	24·6		24·2		24·6	
Butyro refractometer degrees of mixed fatty acids at 25°C. ..	53·2		53·5		54·0	
Iodine value of liquid fatty acids ..		132·7		127·2		126·3

tained 51% of oil. The expressed oil had a faint yellow colour and had the following constants :—

Specific gravity	..	0·9187
N_D 20°C	..	1·4731
Acid value	..	1·4
Iodine value (Hanus)	..	110·4
Saponification value	..	189·3
Acetyl value	..	9·8
Unsaponifiables %	..	1·73
Saturated acids %	..	12·2
Unsaturated acids %	..	81·2

By the ester-fractionation procedure the oil contained the following acids: Linoleic 35·2, oleic 46·0, palmitic 7·3, stearic 4·4, arachidic 0·4 and lignoceric 0·04 by weight per cent.

A specimen of Russian oil was more recently examined by Rudakov and Belo-poleski.[9] The oil was from Siberia. By a combination of Bertram's oxidative process for the total saturated acids with the Kaufman thiocyanogen method for estimating the three unsaturated acids, they reported 15·8% of saturated acids and 37·5% oleic acid and 46·7% linoleic acid by weight of component fatty acids.

The latest examination of the oil was attempted by Hilditch and co-workers.[10, 11] After determination of physical constants and component acids, they tried to establish the glyceride structure by the progressive hydrogenation to several degrees of increasing saturation followed by subsequent analysis of each hydrogenated product (Details given in the glyceride section).

For the present investigation the sample of oil was obtained by cold pressing in the laboratory and had the following constants :

Specific gravity, 28·5°C	..	0·9230
Saponification value	..	190
Acid value, mgms of KOH	..	0·20
Iodine value	..	106·50
Hehner value	..	95·80
Non-saponifiables %	..	0·30

The saturated acids were determined by Hilditch and Priestman's[12] modification of Bertram's procedure.[13] Thus 7·421 g. of oil on oxidation yielded 1·0808 g. of fatty acids of iodine value 0·00 with a mean molecular weight of 268·1. This corresponds to

14·55% on weight of oil. Since the component acids of sesame oil are principally palmitic and stearic in the saturated series with oleic and linoleic in the unsaturated series (previous references), calculation of the fatty acids as palmitic and stearic in the saturated part and oleic and linoleic in the unsaturated part will give rather an accurate idea of the component acids and gives 9·03% palmitic, 5·57% stearic, 45·9% oleic and 38·2% linoleic by weight of the oil if it is considered to be a mixture of the glycerides alone. This, of course, entails some error since the oil contains about 0·3% of unsaponifiables also which being relatively small may be ignored without entailing much error. The glyceride structure of sesame oil does not seem to have been investigated in detail excepting for GS_3 and so a detailed study was undertaken in this investigation.

Hilditch and co-workers[10] have estimated the fully saturated components of a sample of sesame oil. The oil had the composition: palmitic 9, stearic 4, arachidic 1, oleic 46 and linoleic 40 per cent by weight of the component acids. Progressive hydrogenation of the oil showed that the oil which contained little or no fully saturated component, has only 69% of the tri-C_{18} glycerides. The fully saturated acids must therefore be present wholly as mono-saturated (mainly palmito-or stearo-di-"oleins") and over two-thirds of the fat may consist of mixed oleo-linoleins.

In the present investigation 7·4210 g. of oil on oxidation gave 2·1468 g. of bicarbonate insoluble portion with a saponification value of 361·30.[14] This corresponds to 14·67% of GS_2U (details shown in experimental part) and 18·36% GSU_2. GS_3 has been found to be present in traces only by a preliminary oxidation.

Thus the specimen of oil was found to contain by molecules:

GS_3	..	traces
GS_2U	..	15·10 per cent
GSU_2	..	17·5 „
GU_3	..	67·2 „

EXPERIMENTAL:—

PHYSICAL CONSTANTS

1. Density at 28·5°C .. 0·9230

2. Saponification value:—2·3010 g. of oil required 15·60 ml. of N/2 alcoholic potash for saponification. Hence saponification value is 190·01 mg. of KOH. In the duplicate determination

2·810 g. of oil required 14·10 ml. of N/2 alcoholic potash. Hence saponification value is 190·05 mg.

3. Acid value :—10·520 g. of oil required 0·075 ml. of semi-normal potassium hydroxide for neutralisation of free fatty acids. Hence acid value, $\frac{0{\cdot}075 \times 28{\cdot}05}{10{\cdot}520}$ or 0·20 mg. of KOH.

4. Iodine value (Hanus) :—(a) 0·1390 g. of oil absorbed iodine to the equivalent of 15·85 ml. of N/10 sodium thiosulphate. Hence iodine value is 106·49.

(b) 0·1930 grm. of oil absorbed iodine to the equivalent of 16·20 ml. of N/10 thiosulphate. Iodine value, 106·51.

5. Unsaponifiables :—10·1250 g. of oil gave 0·0303 g. of unsaponifiables. Hence percentage of unsaponifiables is 0·2995.

6. Hehner value :—10·125 g. of oil gave 9·6940 g. of water insoluble fatty acids. Hehner value is 95·80.

Solidifying point of the mixed fatty acids or titre test = 22°C.

7. Setting point of oil — 5°C.

8. Melting point of oil 0°C.

Saturated Acid Contents and Glyceride Components

7·4210 g. of the neutral oil was accurately weighed into a 400 ml. round bottomed flask and dissolved in 148 ml. of pure and dry acetone and oxidised with powdered potassium permanganate. 66 g. of permanganate was used up for oxidation. The oxidation product was decomposed with powdered sodium bisulphite and 33% sulphuric acid as in previous cases and the oxidation products extracted to completion with ether. The ether extracts were combined together and washed with a small quantity of water. The ether extract was then washed with 25 ml. of a saturated solution of sodium bicarbonate followed by an equal volume of water to remove bicarbonate soluble portions contained in the ether extract. This operation was repeated two more times, each time followed with distilled water. The bicarbonate and aqueous washings were collected together and immediately acidified and ether extracted.

The ether extract after washing with bicarbonate to remove free acidic products contains a mixture of GS_3, GS_2A and GSA_2.

A preliminary oxidation of 5·05 g. of oil gave only 0·0013 g. of tri-saturated glycerides showing thereby that the fully saturated glycerides are nearly absent. So the ether extract contains a mixture of GS_2A and GSA_2 together with negligible amounts of GS_3. The ether extract was dried with anhydrous magnesium sulphate and transferred quantitatively to a weighed clean and dry flask. Ether distilled off and residue of mixture of glycerides heated to constant weight under reduced pressure. The mixture of glycerides weighed 2·1460 g. It required 27·65 ml. of 0·500 N alcoholic potash for saponification. Hence saponification value is 361·30.

The liquid after finding out the saponification value was warmed on the water bath to remove alcohol. The preliminary bicarbonate and aqueous washings were collected together, acidified and ether extracted. The ether was distilled off and the residue added to the above residue after saponification. The glycerides were saponified once more with excess of potassium hydroxide and the potassium soap of the fatty acids decomposed into free fatty acids with dilute sulphuric acid by warming on the water bath. After cooling, the saturated acids were extracted to completion with ether and ether distilled off.

The residue of fatty acids were dissolved in 296 ml. of hot water and an excess of ammonium hydroxide and heated until the fatty acids completely dissolved. 44·5 ml. of 10% ammonium chloride solution and an excess of 15% magnesium sulphate were added. The saturated fatty acids precipitated as their magnesium soaps. After cooling the precipitate was filtered off and decomposed by heating with slight excess of dilute sulphuric acid and the precipitation of magnesium soaps repeated as before, cooled, filtered and the precipitate returned to the original flask in which the Bertram separation was carried out and decomposed as before by heating with dilute sulphuric acid. After cooling the solution was transferred to a 500 ml. separating funnel and extracted to completion with ether. The ether extracts were combined together and washed with distilled water to remove mineral acid. The ethereal solution of fatty acids was dried over anhydrous magnesium sulphate. Solvent ether distilled off and the mixture of fatty acids which were left behind heated to constant weight at 105°C under reduced pressure.

The saturated acids weighed 1·0808 g. It showed no iodine absorption proving that it is fully saturated and no unoxidised portion of oil was contained in it.

The 1·0808 g. of acid required 8·05 ml. of 0·5000 N alcoholic potash for neutralisation. Hence acid value = 209·3. This corresponds to a mean molecular weight of $\frac{56 \cdot 1}{0 \cdot 2093} = 268 \cdot 1$.

CALCULATION :—

1·0808 g. of saturated acids of iodine value 0·00 is given by 7·4210 g. of oil, corresponding to 14·6% on the weight of the oil. The saturated acids have a mean molecular weight of 268·1.

To arrive at the proportion of "C_{16}" and "C_{18}" saturated acids, the difference between the mean molecular weight of the mixed saturated acids and the molecular weight of palmitic acid is 264·7 — 254·0 = 10·70. The difference between the molecular weights of stearic and palmitic acids is 28. For a difference of 28 in the molecular weights the percentage of stearic acid is 100. The total percentage of saturated acids in sesame oil is $\frac{1 \cdot 0808}{7 \cdot 421} \times 100 = 14 \cdot 60$.

The percentage of C_{18} acid is therefore, $14 \cdot 60 \times \frac{10 \cdot 7}{28} = 5 \cdot 57$, and of C_{16} acid is 9·03%.

This corresponds to weight on oil and to convert it into weight per cent of fatty acids, it has to be multiplied by 100/95·8 since 95·8 is the Hehner value and on converting into weight per cent of fatty acids, the figures for palmitic and stearic become 9·426 and 5·8. On conversion into moles per cent the values for palmitic and stearic become 10·27 and 5·7 molecules respectively.

CALCULATION OF UNSATURATED ACID CONTENT

The oil was tested for the presence of linolenic acid by the hexabromide derivative. But the bromide obtained by brominating the oil in dry chloroform with bromine in chloroform melted only at 114°C even after five crystallisations from acetic acid. If linolenic acid was present linolenic hexabromide of melting point 184°C should have been obtained. Hence the oil can be taken to be free of linolenic acid.

The percentage of saturated acids in the oil is 14·60 and that of saturated acids in combination as glycerides is $\frac{14 \cdot 60\ (264 \cdot 7 \times 3 + 38)}{264 \cdot 7 \times 3}$ or 15·30. The iodine value of the oil is 106·50 and this is due to the unsaturated glycerides alone. The

iodine value of the unsaturated glycerides is $\frac{106 \cdot 50}{(100 - 15 \cdot 3)} \times 100$ or 125·70. The iodine value of triolein is 86·2 and of trilinolein is 173·6. Hence the percentage of trilinolein, when the iodine value of the mixture of the two is 125·7, comes to $\frac{(125 \cdot 7 - 86 \cdot 2 \times 84 \cdot 7}{173 \cdot 6 - 86 \cdot 2}$ or 38·28.

That of triolein is $84 \cdot 7 - 38 \cdot 28 = 46 \cdot 42\%$.

Hehner value of the oil is 95·80 and the percentage of saturated acids is 14·60%. Hence if the oil contains no water soluble acid (previous workers), the percentage of unsaturated acids $= 81 \cdot 2\%$. Consequently the percentage of oleic acid will be $\frac{282 \times 3 \times 46 \cdot 42}{(282 \times 3) + 38}$ or 44·41%.

Similarly that of linolenic acid will be 36·8%.

Converting into weight per cent of the fatty acids, oleic forms 46·55 and linolenic 36·97 respectively of the fatty acids.

On conversion into moles per cent the unsaturated acids form 84·1% by molecules of the total acids comprising of 45·9 per cent of oleic and 38·2% of linoleic by molecules.

Glyceride Composition

The molecular proportion of saturated and unsaturated acids are in the ratio of 15·9 to 84·1.

The saponification value for GS_2A is $\frac{4 \times 56 \cdot 1 \times 1000}{755 \cdot 4} = 297 \cdot 3$ and for GSA_2 is 413·4.

The above figures are based on the observation that the ratio of the saturated acids is the same in the two types.

As a preliminary oxidation with 5·040 g. of oil gave 0·0013 g. of bicarbonate and carbonate insoluble glycerides, i.e., trisaturated glycerides, the absence of fully saturated glycerides in appreciable quantities can be safely assumed.

7·4210 g. of oil yielded 2·1460 g. of the mixture of the two azelao glycerides and the tri-saturated glycerides if any. Since the latter has been found to be negligible the saponification value of 361·3 is due to the mixture of glycerides alone.

Ratio of $\frac{GS_2U}{GS_2A} = \frac{849 \cdot 4}{755 \cdot 4}$

The weight of GS_2A in the mixture is $2 \cdot 146 \times \frac{52}{116 \cdot 1}$

Weight of GS_2U corresponding to this is $2{\cdot}146 \times \frac{849{\cdot}4}{755{\cdot}4} \times \frac{52{\cdot}1}{116{\cdot}1}$

The percentage by weight of GS_2U in the oil

$$= \frac{2{\cdot}146}{7{\cdot}421} \times \frac{849{\cdot}4}{755{\cdot}4} \times \frac{52{\cdot}1}{116{\cdot}1}$$
$$= 14{\cdot}67$$

which on conversion into moles per cent becomes 15·10.

The molecular percentage of saturated acids has been found to be (vide supra) 15·90, GS_3 0, and GS_2U 14·67. Hence percentage of GSU_2 is $(15{\cdot}9 - 0{\cdot}00) - (15{\cdot}1 \times 2/3) = 17{\cdot}5$.

Thus the specimen contains GS_3 traces, GS_2U 15·10 and GSU_2 17·5 per cent by molecules. The remaining per cent must have existence only as GU_3 and the percentage molecule of GU_3 is 67·27.

Summary

A sample of cold pressed sesame oil has been examined by Bertram's oxidation method for component acids and found to contain palmitic acid 10·2, stearic 5·7, oleic 45·9 and linoleic 38·2 per cent by molecules.

The distribution of glycerides in the sample on investigation by the tentative oxidation method was found to be as follows :—

	GS_3	.. Traces
	GS_2U	.. 15·1
	GSU_2	.. 17·5
and	GU_3	.. 67·3 per cent by molecules.

REFERENCES

1. Lewkowitsch, '*Chemical Technology and Analysis of Oils, Fats and Waxes*' 1921, Volume II.
2. Utz, *Pharm. Hg. Zeitg.*, 1900, 45, 490.
3. Sprinkmeyer and Wagner, *J. Soc. chem. Ind. Lond.*, 1900, 914
4. Lane, *J. Soc. chem. Ind. Lond.*, 1901, 1083.
5. Wijs, *Zeit. Sch. Nahr. Genussm.*, 1902, 5, 1150.
6. Sprinkmeyer and Wagner, *Zeit. Sch. Nahr. Genussm.*, 1905, *10*, 347.
7. Hefter, *Fette u. Oele.*, 1908, II, 274.
8. Jamieson and Baughman, *J. Amer. chem. Soc.*, 1924, *46*, 775.
9. Rudakov and Belo-poleski, *Maslob. Shir Delo.*, 1931, No. 2-3, 60.
10. Hilditch, Ichaporia and Jasperson, *J. Soc. chem. Ind. Lond.*, 1938, 57, 363.
11. Hilditch and Riley, *J. Soc. chem. Ind. Lond.*, 1945, *64*, 204.
12. Hilditch and Priestman, *Analyst*, 1931, *56*, 554.
13. Bertram, *Chem. Weekblad.*, 1927, *24*, 226.
14. Kartha, Venkitasubramanian and Menon, *Proc. Indian Acad. Sci. A*, 1945, *21*, 228.

FOOD OF THE RAINBOW SARDINE (*DUSSUMIERIA ACUTA* CUV. AND VAL.)

BY

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INTRODUCTION

Delsman (1925, 1929) expressed the belief "that there is probably only one species of *Dussumieria,* and not two, as assumed by Day and Weber and Beaufort after Bleeker's example," and assigned the species occurring in the Java seas to *Dussumieria hasseltii.* His identification was based on the occurrence of 52 to 56 scales in the lateral line, in which it differs from *D. acuta.* But he confessed that it was not possible to distinguish the two species since the scales which afford the most conspicuous difference between them are extremely deciduous. The same difficulty has been experienced by Devanesen and Chidambaram (1948) in distinguishing the two species occurring in the Madras waters. However, it has been found possible for the author, from observations made on a number of fresh specimens taken from the nets when they arrived ashore, to distinguish from *D. hasseltii,* a number of specimens as *D. acuta* with scales in the lateral line numbering less than 42, a feature characteristic of *D. acuta.*

'*Modukandai,*' which is the Tamil name for the species, occurs about a mile from the coast and are caught in enormous numbers from July to December. In January they are found in very large numbers, while in May and June they are very rare.

Very little is known about the food of this fish. Devanesan and Chidambaram (1948) make a reference to the food of the rainbow sardine in the course of an account of the common food fishes of the Madras Presidency, and Chacko (1949) has given a list of organisms from the stomach contents of *D. hasseltii* off the Gulf of Manaar. Except these, the author has not come across any detailed account of the food of this fish. Therefore, in this paper a descriptive account of the food of *D. acuta* obtained by a volumetric analysis of the stomach contents of 233 specimens collected in a period of 24 months is presented and the fluctuations in the chief items of

diet in relation to the availability of food in the environment as well as in relation to the growth of the fish are discussed.

The methods followed in the analysis of the stomach contents were those described by the author elsewhere (Vijayaraghavan 1951).

The largest specimen examined was 8·9 inches and the smallest 3·1 inches long. For the purpose of this study the fishes examined were divided into two size groups, the five inches size group which included all those which were five inches and above and the four inches group which included those which were below five inches.

Dussumieria acuta (Cuv. and Val.)

Four inches size group.

Both sexes of the fish belonging to this group caught between July and February at different hours of the day were examined and their stomach contents analysed. Table I gives the results of the analysis, and it is observed that the fish subsists chiefly on crustacea. The steady increase in the amount of crustacea consumed from July to October is worthy of note.

Five inches size group.

Fish of this size group obtained from July to April were examined in the same lines as the four inches group. The maximum number was caught during January.

The results of the analysis are presented in Table II. While crustaceans form the major item of diet of the fish, from January onwards it includes small amounts of teleostea in the menu.

Discussion

A study of the fluctuations in the food items in the stomach of *Dussumieria acuta* throws light on the possible correlation of food with growth.

It has been recorded that in July there is a preponderance of 4″ group which by August are more or less equal in number, to the 5″ group. During December there is an increase in the 5″ group and a dwindling of the 4″ group which are totally absent by March. It appears probable that the 4″ group grows into the 5″ group by December and the next size grade is reached only in May when the fish are absent having probably migrated to feeding or spawning areas elsewhere. During these periods of growth the

TABLE I

VOLUMETRIC PERCENTAGES OF THE FOOD COMPONENTS OF 4" SIZE GROUP OF D. ACUTA

Month	July	August	October	December	February
No. of Specimens examined ..	12	9	3	7	3
No. with food contents ..	12	9	1	7	3
Crustaceans ..	26·52	50·88	95·00	10·57	7·99
Penaeus Larvae ..	—	—	90·0	—	—
Lucifer ..	4·58	—	—	·14	—
Other Sergestids ..	1·92	—	—	—	—
Mysidacea ..	1·92	—	—	—	·67
Paguridae ..	8·33	—	—	—	—
Zoea of crab ..	—	·22	—	·14	·33
Megalopa of crab ..	6·27	—	4·0	7·29	·33
Copepoda ..	2·50	16·44	1·0	1·71	3·33
Copepod nauplii ..	—	34·00	—	—	—
Octracoda ..	—	·22	—	0·14	—
Amphipoda ..	—	—	—	·86	·33
Squilla larvae ..	—	—	—	—	·33
Crustacean remains ..	1·00	—	—	·29	2·67
Ophiuroidea ..	·09	—	—	—	—
Bivalve larvae ..	·27	—	—	—	—
Eggs (Teleostean) ..	·33	—	—	—	—
Sagitta ..	—	—	—	3·86	—
Algae ..	·17	8·67	—	—	5·67
Green matter ..	—	·53	—	—	—

TABLE II

VOLUMETRIC PERCENTAGES OF THE FOOD COMPONENTS OF 5" SIZE GROUP OF DUSSUMIERIA ACUTA

Month		July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
No. of specimens examined	..	8	24	30	20	24	23	21	17	20	12
No. with food contents	..	6	21	30	18	24	20	17	9	16	9
Teleosteans	..	—	—	—	—	—	—	3·82	3·21	1·72	15·8
Crustaceans	..	23·33	36·04	27·27	26·49	39·84	41·75	29·67	23·55	14·70	20·34
Acetes	..	—	·91	2·07	12·11	—	—	2·05	—	—	—
Penaeus	..	1·67	·95	9·07	11·22	·71	4·05	—	—	1·13	—
Mysidacea	..	12·83	4·76	11·0	—	7·21	—	·06	14·44	—	—
Lucifer	..	·67	5·43	·40	2·39	6·71	·30	·18	·11	·50	—
Other Sergestideae	..	—	—	—	1·72	—	—	—	—	—	—
Paguridae	..	—	—	1·0	·39	3·33	8·10	—	—	—	·12
Other Anomurans	..	—	—	·77	1·33	—	·90	1·09	—	—	—
Zoea of crab	..	—	—	·07	—	·04	—	—	—	—	—
Megalopa of crab	..	5·50	4·52	5·17	—	7·96	2·00	2·23	1·33	·25	13·22
Crabs	..	—	—	·53	—	—	—	—	—	—	—
Copepoda	..	2·66	1·52	2·40	·44	4·83	·95	3·00	1·56	1·50	2·56

TABLE II (contd.)

Month	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Ostracoda ..	—	·95	1·13	1·11	·17	·90	·35	—	·69	2·00
Amphipoda ..	—	·14	—	—	·54	·25	—	2·0	·06	—
Cumacea ..	—	—	·13	·11	·38	—	—	—	—	—
Phyllosoma ..	—	—	—	—	·04	—	—	—	—	—
Squilla larvae ..	—	14·10	2·53	—	—	19·05	14·18	—	6·82	2·44
Crustacean remains ..	—	2·76	2·10	4·67	7·92	5·25	6·53	4·11	3·75	—
Sagitta ..	5·67	—	—	—	7·86	·50	—	7·44	·25	—
Polychaeta ..	—	—	·90	—	·09	—	—	·22	—	—
Bivalve larvae ..	—	—	·37	·72	·29	·10	·05	—	—	—
Eggs (Teleostean) ..	—	—	·13	—	—	—	—	—	—	—
Madusae ..	—	—	—	—	·04	—	—	—	—	—
Algae ..	·5	·1	1·27	·89	·29	·25	—	·78	·25	·22
Green matter ..	—	—	·73	·50	·71	1·4	·52	—	1·25	·22
Sand particles ..	—	·1	·17	·17	·04	—	·23	·44	·38	·12

fluctuations in the items of diet as found from an examination of the stomach contents are of interest. From July to October when the 4″ group increases its rate of feeding, the crustaceans form the main source of food supply. Again in January when the 5″ group which reaches its maximum strength there is an increase in the crustacean diet. Though teleosteans are also included, they are of a negligibly small amount. Thereafter there is a fall in the rate of feeding till March when once again increase in the teleostean as well as the crustacean items of food is found, probably suggestive of the growth of the fish into the next size grade. It is interesting that the analysis of the above data shows that the fluctuations in the abundance of crustaceans and teleosteans are not correlated with the rise and fall in the consumption of the items of diet.

Summary

1. The stomach contents of the 4″ and 5″ size groups of *Dussumieria acuta* (Cuv and Val) are analysed and the fluctuations in the favourite items of diet are discussed.

2. While fluctuations in the rate of feeding of the fish are probably correlated with its growth, they are not influenced by the fluctuations in its favourite items of diet occurring in the environment.

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REFERENCES

Chacko P. I. 'Food and feeding habits of the fishes of the Gulf of Manaar', *Proc. Indian Acad. Sci.*, Vol. XXIX, 1949.

Delsman. H. C., 'Fish eggs and larvae from the Java sea,' *Treubia*, Vol. VI, 1925.

Delsman, H. C. 'Study of Pelagic fish eggs', *Fourth Pacific Sci. Cong.* May-June 1929.

Devanesan, D. W. and Chidambaram, K., 'A Madras Government Publication', 1948, p. 17.

Vijayaraghavan, P., 'Food of the Ribbon fishes of Madras' *J. Madras Univ.* B. Vol. XXI, 1951.

SEED-BORNE FUNGI AND LOSS OF SEEDLING VIGOUR IN COTTON

BY

C. S. VENKAT RAM

INTRODUCTION

A survey of the internal microflora of cotton seeds collected from the Madras Presidency from 1946-1949 showing preponderance of species of *Aspergillus, Rhizopus, Penicillium* and *Alternaria,* in the order mentioned, has been reported earlier (Venkat Ram, 1950). Preliminary experiments mentioned therein indicated that these internal seed-borne fungi when present in soil were capable of reducing germination in cotton. It is an established fact that seeds harbouring infection are important sources of disease transmission (Bolley, 1910, 1912, 1913; Crawford, 1923; Dastur, 1932, 1934; Drechsler, 1923; Elliot, 1923; Ganguly, 1947; Henry, 1924; Kenderick, 1931, 1934, 1944; Kulkarni, 1934; Leukel and Martin 1943; Machacek and Greaney, 1938; Mead, 1943; Mitra, 1935; Orton, 1931; Pethybridge and Lafferty, 1918; Ray and McLaughlin, 1942; Rudolph and Harrison, 1945; Simmonds, 1930; Snyder, 1932; Stakman, 1920; Taubenhaus and Ezekiel, 1932) and recently Tervet (1945) was able to demonstrate the importance of the seed-borne saprophytes in inducing disease reactions and reducing vigour in the germinating soyabean seedlings. It must, however, be stated that the factor inducing disease reaction in germinating seedlings by seed-borne organisms is largely determined by the soil environment, in its *modus operandi.* The problem of investigating seed microfloral complex actively participating in the predisposition of the seed to disease incidence by imparing vigour is of obvious importance in devising feasible and effective methods of controlling seed-borne organisms.

The present investigation was designed primarily to evaluate the role of the seed-borne fungi in influencing seedling vigour of cotton and some of the important observation are discussed here.

MATERIALS AND METHODS

All fungi under investigation were the isolates from the earlier studies (Venkat Ram, 1950). Cotton seeds of the different varie-

ties were obtained from the Director of Agriculture, Madras, unless otherwise stated; all seeds being acid delinted and surface sterilized prior to experimentation. pH determinations were made with the help of a Beckman pH meter using a glass electrode.

A similar procedure to that of Tervet (1945) was followed in determining the 'extent role' of the fungi in influencing seedling vigour. The isolates were cultured on potato dextrose broth in 250 ml. conical flasks and after 15 days incubation at 28°-30°C, the mats were filtered off to obtain the filtrate. Spore suspension of the organisms was obtained by shaking sterile water in 250 ml. conical flasks containing 10 day old colonies of these fungi on potato dextrose agar medium. All seeds were germinated in earthenware pots containing acid washed sand as substratum; the pots were incubated under glasshouse conditions, watered daily and germination counts taken upto 15 days. Index of seedling vigour was compared by taking the dry weights of seedlings, the latter being achieved as per recommendations of A.O.A.C.

Experimental

A. *Seed-borne fungi and seedling vigour*

Experiment I : Cotton seeds of Karunganni I. variety (*Gossypium arboreum*) were soaked in filtrate and spore suspension of the various seed-borne fungal isolates in sterile 10 cm. Petri dishes for 24 and 48 hours at Laboratory Room temperature 28°-30°C. after which they were germinated taking into account 150 seeds from each lot in three replicates. Seeds soaked in sterile distilled water for similar periods and germinated, provided the control. Results are given in Table I and Plate I, fig. 5.

It was seen that a number of fungal forms studied, reduced germination and seedling vigour to varying degrees. The pathogenic manifestations of *Corticium solani* was noteworthy in that, whereas presence of the fungus in the form of mycelial suspension completely inhibited germination, the filtrate had no such injurious effect. *Aspergillus niger* behaved similarly with regard to its filtrate and spore suspension, both impairing germination to a considerable extent. The two forms *Aspergillus fumigatus* and *Aspergillus nidulans* while showing only slight impairment when present as spore suspension, completely inhibited germination when seeds were soaked in their filtrates.

TABLE I

SHOWING INFLUENCE OF SOAKING IN FILTRATE AND SPORE SUSPENSION OF VARIOUS FUNGAL ISOLATES ON GERMINATION AND SEEDLING VIGOUR OF COTTON

Fungal inoculum	Isolated from	Period of soaking in hours	Cotton seeds soaked in Spore suspension			Cotton seeds soaked in Filtrate	
			Percentage germination	**Dry weight of seedling in mg.	pH	Percentage germination	**Dry weight of seedling in mg.
Fusarium vasinfectum	Exterior of cotton seed and infected seedling	24	72	214	6·2	72	237
		48	45	134		70	213
Fusarium moniliforme	Diseased cotton seedling	24	75	205	—	77	205
		48	50	120		75	190
Macrophomina phaseoli	Interior of cotton seed	24	63*	192	7·3	85	190
		48	43*	122		84	187
Corticium solani	Cotton seedling	24	0*	—	6·7	88	233
		48	0*	—		82	192
Aspergillus ustus	Interior of cotton seed	24	75	226	6·9	88	163
		48	74	101		88	155
Aspergillus niger	Do.	24	24	136	2·6	18	233
		48	0	—		0	—

Aspergillus fumigatus	Do.	24	70	209	3·2	0	—
		48	50	145		0	—
Aspergillus nidulans	Do.	24	88	200	5·0	4	—
		48	72	152		0	—
Aspergillus flavus	Do.	24	89	188	4·3	86	269
		48	80	153		38	258
Aspergillus terreus	Do.	24	88	235	6·1	82	252
		48	80	147		62	246
Penicillium sp. I	Do.	24	84	196	5·4	87	231
		48	81	127		87	229
Penicillium sp. II	Do.	24	82	198	4·1	85	240
		48	62	132		5	—
Rhizopus nodosus (Strain A)	Do.	24	62	182	8·2	55	224
		48	12	124		48	218
Rhizopus nodosus (Strain B)	Do.	24	60	194	3·0	4	—
		48	4	—		0	—
Control		24	92	238	7·0	92	238
		48	89	233		89	233

* Mycelial suspension

** Mean of 3 readings of six seedlings each

Soaking seeds in the spore suspension of the two strains of *Rhizopus nodosus* was found to decrease germination percentage considerably when the incubation period of soaking increased from 24 to 48 hours. Somewhat analogous to this was the reaction shown by the filtrate of *Penicillium* sp. II.

Filtrate of *Rhizopus nodosus* (strain B) reduced germination to a very marked extent as when compared to Strain A, thus providing further proof of their physiological dissimilarities, reported in the earlier communication (Venkat Ram, 1950).

As regards the influence of these isolates on seedling vigour, the majority of the forms impaired vigour as evidenced by decrease in dry weight of the seedlings produced from seeds soaked in either spore suspension or the filtrate, as when compared to the control. In every case increase in period of soaking from 24 to 48 hours impaired seedling vigour to a very marked extent. The form *Aspergillus ustus* was particularly severe in reducing vigour, seeds soaked in the filtrate as well as in spore suspension for 48 hours producing seedlings of almost one half the dry weight of that in the control. In general, soaking in spore suspension contributed considerably more to reduction in dry weight of the seedlings than soaking in filtrates, this being very marked when seeds were soaked in spore suspension for 48 hours.

When the capacity of a particular fungus to reduce germination, in either its spore or filtrate form, was equated with ability to impair seedling vigour, no definite correlation was found to exist. It would seem that three distinct types of disease reactions produced by the various isolates could be differentiated. (1) Types reducing germination percentage without impairing seedling vigour of germinating seedlings, (2) Forms impairing germination as well as vigour and (3) Those allowing normal germination but impairing vigour. The severest manifestation of the second type of disease reaction was noticed in the case of *Aspergillus niger, A. fumigatus, A. nidulans* and *Rhizopus nodosus* (Strains A and B), all being seed-borne fungi of cotton (Table I and Plate I, fig. 5).

Experiment II: Since most of the forms induced strong acid reaction in their growth substrate (Table I), the possibility of attributing the toxicity of their filtrates in germination and vigour to low pH was envisaged. To clarify this point, cotton seeds of K.I. variety were soaked in sterile water blanks adjusted to various hydrogen-ion concentrations ranging from pH 2·5 to 8·5 in sterile

Petri dishes for 24 and 48 hours and germinated similarly. Results are incorporated in Table II.

TABLE II

SHOWING EFFECT OF SOAKING SEEDS IN DISTILLED WATER AT DIFFERENT pH LEVELS ON GERMINATION AND VIGOUR OF COTTON

pH of the medium	Seeds soaked for 24 hours		48 hours	
	Percentage germination	Dry weight of seedlings in mg.	Percentage germination	Dry weight of seedlings in mg.
2·5	52	233	48	231
3·0	88	231	84	227
3·5	89	237	83	228
4·0	90	234	86	230
5·0	89	235	85	229
6·0	88	237	85	230
7·0	90	232	84	226
8·0	90	232	85	230
8·5	90	228	82	217

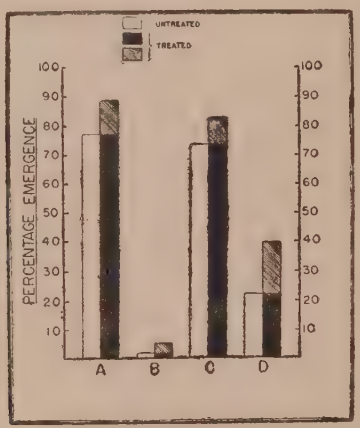
It is seen from Table II that no correlation existed between pH of the medium in which seeds were soaked and vigour of the seedlings. The very high hydrogen-ion concentration level of 2·5 pH reduced germination considerably but pH of the medium at and above 3·0 had no detrimental effect.

It is, therefore, clear that pH of the filtrate had no part in impairing germination and vigour of cotton seeds and that some other factor, probably biotic or chemical, operated in causing disease reaction.

Experiment III : Thus in deciding the germination and vigour of a seed lot, much would depend on the presence of seed-borne fungal types and their probable role in seed deterioration both in storage and germination. However, application of fungicide to stored seeds with a view to warding-off fungal infection from without did not ameliorate seed viability (Robertson *et al*, 1939; Arndt, 1946; Venkat Ram, 1950) although Simpson (1946) has stated the beneficial effects of fungicidal application to stored seeds in germination. It is, therefore, probable that most of the damage done by

seed-borne fungal organisms is perhaps by their activity in soil. To elucidate this point the following experiment was conducted.

Fuzzy and acid delinted cotton seeds of H.I. variety *(Gossypium herbaceum)* with initial moisture contents of 9 and 12·5 per cent, under storage in sealed containers for 9 weeks at 28-30°C, untreated and treated with 2 per cent Agrosan G prior to storage, were germinated in garden soil in lots of 100 under glass house conditions. Parallel experiments with batches of 100 seeds, freshly collected and known to contain only a small percentage infection, were sown separately in sterile and non-sterile soils after previous delinting and surface sterilization. Results are presented in Text-fig. I and Plate I, fig. 6 with the following conclusions.



Text-Fig. 1. Showing the percentage emergence of H.1 (*Gossypium herbaceum*) seeds—fuzzy and delinted, untreated and treated with 2 per cent. Agrosan G—in soil after storage for 9 weeks at 28°-30° C.

A Delinted seeds of 9 per cent. moisture content.
B Delinted seeds of 12·5 per cent. moisture content.
C Fuzzy seeds of 9 per cent. moisture content.
D Fuzzy seeds of 12·5 per cent. moisture content.

Germination in soil assessed on percentage emergence basis was markedly greater in seed lots treated with the fungicide.

The difference between germination percentages of treated and untreated seeds in soil was much more pronounced in lots which showed a greater loss in viability in storage. The fungicidal treatment to such seed lots seemed to have a marked ameliorative effect on seed germination in soil—probably by bringing about the control of fungal forms.

Plate I, fig. 6 shows the striking difference in germination and vigour of seedlings when sown in sterile and non-sterile soils, the actual difference in percentage germination and vigour of seeds sown in sterile and non-sterile soil being in the ratio of 4 : 3.

B. *Germination and infection of light and heavy cotton seeds*

Chester (1938) investigating germination of acid delinted cotton seed which were separated into light and heavy fractions on the basis of their specific gravity relative to that of water, stated the superiority of heavy seeds. Later Arndt (1945) employing the same method studied germination and infection in cotton seeds of American varieties and indicated higher percentage infection and lesser germination in light than in heavy seeds. The following studies were undertaken to evaluate the relationship existing between germination and infection in light and heavy cotton seeds of indigenous varieties collected from various sources in South India. Seeds kept under different storage conditions were experimented with, the technique for culturing and germination being the same as described by Venkat Ram (1950). Results are given in Table III.

Most of the widely cultivated varieties in South India contained floaters showing very poor germination.

In contradistinction to the low germination and high percentage infection of floaters seen in the types belonging to *Gossypium arboreum,* the variety H-1 (*Gossypium herbaceum*) contained a high percentage of floaters showing good germination coupled with low infection.

Higher percentage infection in floaters depended on longevity in storage period and on the intrinsic nature of the seeds themselves, probably being determined by the environmental conditions governing the formation of the seeds on the plants, prior to maturity.

Since a high percentage of floaters with poor germination contained appreciable number of seeds showing fungal infection, the probable role of fungi in the idiosyncratic development of floaters is envisaged.

C. *Soil conditions and parasitism of Rhizopus nodosus* Namysl.

The isolate *Rhizopus nodosus* (Strain A)—first time recorded from within the cotton seed and known to reduce germination considerably (Venkat Ram, 1950)—was further studied for its pathogenic interactions in relation to soil moisture content; previous studies showed this strain to behave differently in respect to variation in soil pH and the nature of the fungal inoculum employed.

TABLE III

SHOWS GERMINATION AND INFECTION OF COTTON SEEDS SEPARATED INTO TWO FRACTIONS 'FLOATERS' AND 'SINKERS' IN RESPECT OF THEIR SPECIFIC GRAVITY RELATIVE TO THAT OF WATER

Seed variety	Source and year	Storage period in weeks	Percentage germination		Percentage infection	
			Floaters	Sinkers	Floaters	Sinkers
Karunganni I. (*Gossypium arboreum*)	Experimental plot, Univ. Bot. Lab. 1946	4	15	82	68	20
K. 4706 (*G. arboreum*)	Do. 1947	1	2	80	49	8
		6	4	80	75	23
Karunganni I. (*G. arboreum*)	Do. 1948	6	6	75	69	18
Guntur I. (*G. arboreum*)	Agric. Res. Officer, Narsaravpet, S. India, 1947	10	16	90	23	21
		16	13	83	56	22
Hagari I. (*G. herbaceum*)	Agric. Officer, Hagari, S. India, 1947	12	54	96	7	4
		18	50	95	15	4
Karunganni I. (*G. arboreum*)	Cotton specialist, Coimbatore, S. India, 1948	12	12	81	29	14
Nandyal 14. (*G. arboreum*)	Agric. Officer, Nandyal, S. India, 1948	12	18	90	27	19

The experimental layout was similar to one described earlier (Venkat Ram, 1950), soil moisture levels studied being 50, 55, 60, 65, 70 and 75 per cent. Results are incorporated in Table IV.

TABLE IV

SHOWING PERCENTAGE GERMINATION OF COTTON SEEDS IN UNSTERILIZED SOIL ADJUSTED AT DIFFERENT MOISTURE LEVELS AND INOCULATED WITH *RHIZOPUS NODOSUS* (STRAIN A)

Moisture content of soil	Percentage germination of seeds (15 days after sowing)		Total number of seedlings infected (after 30 days' incubation)	
	Inoculated series	Control	Inoculated series	Control
50%	32	68	4	1
55%	34	69	5	2
60%	44	73	5	0
65%	50	75	6	0
70%	64	76	8	0
75%	65	75	10	0

Lowering of the soil moisture content reduced germination percentage considerably in the inoculated series. Antipode to this was the increase in percentage post-emergence infection of seedlings in higher soil moisture levels. Infection of the germinated seedlings was manifest by the attack of the cotyledons by the fungal mycelium, the latter showing profuse growth and sporulation which extended on to the soil (Plate I, figs. 1 and 4). In certain cases the fungus attacked the cotyledons soon after emergence resulting in decay of the seedling (Plate I, figs. 2 and 3). It was interesting to note that decrease in soil moisture level from 75% to 50% reduced germination by one half (Table IV).

Discussion

Some of the aspects detailed in these studies bring into prominence the importance of further investigations in the problem of seed deterioration and loss in seedling vigour due to seed-borne fungi—mainly saprophytes, in this country. Work done elsewhere by Muth (1904), Johann (1928, *et al*, 1931), Leukel and Martin (1943) and Tervet (1945) emphasise the importance of saprophytes in understanding loss in seedling vigour. Indeed, Leukel and Martin (1943) stated that reduction in stand of sorghum seedlings

due to dusting with spores of *Penicillium oxalicum* was much more severe than that due to damping-off by *Fusarium moniliforme,* a virulent pathogen.

Internal infection in seed is limited, being mainly influenced by the environmental factors affecting seed maturity and storage conditions, whereas infection exterior to the seed by air-borne contaminants is common. Recently Subramanian (1950) and Zachariah, (1950) studying various South Indian soil types showed the ubiquity and perennation of the common seed-borne fungi, isolated by the author (Venkat Ram, 1950), in these soils. Ability of these to cause disease reaction in cotton (Table I.) has been demonstrated clearly, extending from the milder forms to the severest manifestations of disease incidence shown by *Aspergillus niger, A. fumigatus, A. nidulans* and *Rhizopus nodosus.* All the fungal isolates investigated showed either one of the three types of disease reactions i.e., ability to reduce germination, ability to impair seedling vigour or both, thereby emphasizing their importance when present as infection foci either in seed or soil. Evidence in support of these findings is provided by somewhat similar results obtained in the case of soyabean by Tervet (1945), in the case of paddy by Ganguly (1947), in the case of Sorghum by Leukel and Martin (1943), in cereals by Dastur (1932), Mitra (1935) and Mead (1943), in wheat by Bolley (1910, 1912, 1913), Stakman (1920), Christensen and Stakman (1935), Drechsler (1923), Henry (1924) and Simmonds (1930) and in flax by Pethybridge and Lafferty (1918).

By possible control of seed and soil-borne organisms, fungicidal application to seeds prior to storage is advocated to ensure better germination (Text-fig. 1) although this does not ameliorate seed viability (Venkat Ram, 1950). These results are in agreement to findings of Simpson (1946) and Arndt (1946). Sterilized soil as a substratum was positively superior to unsterilized soil in germination tests (Plate I, fig. 6) indicating that perhaps the microflora of the unsterilized soil interfered with germination and reduced vigour as already pointed out (Table I). In this connection mention must be made of the work of the Machack and Wallace (1942) on superiority of unsterilized soil as a medium of test in germination of cereals. It is possible that the cereals employed by them as indicator plants behaved differently to cotton which is more susceptible to fungal infection. Moreover the successful use of unsterilized soil as a medium for germination is restricted to availability of suitable conditions viz. temperature of 20°C etc., which are basic prerequisites, while it is quite simple to obtain sterilized soil. It is also signi-

ficant to observe that Machacek and Wallace (1942) likewise recommend fungicidal pre-treatment of seeds to ensure better germination and stand, as observed by the author, when unsterilized soil is employed as a medium for germination tests.

Pre-determination of germination capacity of the 'light' fraction in every cotton seed lot would be essential if skrips in rows and poor stand are to be avoided, since the 'light' fraction of the common varieties grown in Southern India showed extremely poor germination in contradistinction to the American types studied by Arndt (1945). Results of these studies indicate that germination and infection of 'light' and 'heavy' cotton seeds in different varieties largely depend on either the intrinsic nature of the seeds themselves or the environmental factors governing seed maturity.

Virulence of *Rhizopus nodosus* Namyslowski in reducing germination and infecting cotton seedlings was considerably influenced by soil moisture, lower levels enhancing disease incidence (Table IV); soil pH and the nature of the inoculum influencing pathogenic reactions of this fungus has already been discussed in earlier studies (Venkat Ram, 1950). These generalized results point out to the possibility of controlling the activity of the seed-borne fungi, also present in soil, concerned in lowering germination and vigour of seedlings, by proper soil management.

The mechanism by which the saprophytic fungi reduce germination and vigour has not been fully understood. According to Raistrick and Clark (1919), Johann *et al* (1931) and Diachun (1939), it is due to secretion of acid by the saprophytes although results obtained here (Table II) controvert this statement, no disease reaction being found to be attributable to pH. It is possible that secretion of enzymes by the fungi might have played a part in causing disease as postulated by Leukel and Martin (1943) and Tervet (1945) and only further work will show the validity of this postulate.

Summary

The part played by seed-borne fungi in influencing germination and seedling vigour of cotton was evaluated with a view to determining the microfloral complex predisposing seed to disease incidence when germinated in soil.

1. Soaking seeds in the spore suspension and filtrate of various isolates resulted in poor germination and reduced seedling vigour, ranging from very mild forms of disease reaction to the severest manifestations of infection.

2. The mechanism of infection due to the fungal forms tested does not seem to be due to secretion of acids by the fungi.

3. Application of fungicide to the seed prior to storage is recommended to ensure better germination.

4. Sterilized soil as a medium for germination tests is advocated since the microbiological factors of unsterilized soil were found to impair germination and vigour.

5. Possibility of controlling pathogenic activity of seed-borne fungi is suggested by proper management and modifications of soil conditions.

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REFERENCES

A. O. A. C. (1945) "Official and Tentative Methods of Analysis, Washington D.C.

Arndt, C. H. (1945) "Viability and infection of light and heavy cotton seeds". *Phytopath.* 35 (10): 747-753.

Arndt, C. H. (1946) "The internal infection of cotton seed and loss of viability in storage". *Phytopath.* 36 (1): 30-37.

Bolley, H. L. (1910) "Seed disinfection and crop production methods and types of machinery used". *N. Dakota Agr. Exp. Sta. Bull.* 87.

Bolley, H. L. (1912) "Root diseases of cereals and soil studies". *Rept. of Botanist and State Seed Commissioner. Twenty-second Ann. Rept. N. Dakota Agr. Exp. Sta.* 3-40.

Bolley, H. L. (1913) "Wheat: Soil troubles and seed deterioration". *N. Dakota Agr. Exp. Sta. Bull.* 107.

Chester, K. S. (1938) "Gravity-grading, a method for reducing seed-borne diseases in cotton." *Phytopath.* 28: 745-749.

Crawford, R. F. (1923) "Fungi isolated from the interior of cotton seed". *Phytopath.* 13: 501-503.

Christensen, J. J. and Stakman. E. C., (1935) "Relation of *Fusarium* and *Helminthosporium* in barley seed to seedling blight and yield." *Phytopath.* 25: 309-327.

Dastur, J. F. (1932) "Foot-rot and 'Black Point' diseases of wheat in the Central Provinces". *Agric. and Live Stock in India,* 2: 275-282.

Dastur, J. F. (1934) "Cotton anthracnose in the Central Provinces". *Indian J. agric. Sci.* 4 (1): 100-120.

Diachun, S. (1939) "The effect of some soil factors on *Penicillium* injury of corn seedlings." *Phytopath.* 29: 231.

Drechsler, C. (1923) "Some graminicolus species of *Helminthosporium*. *J. agric. Res.* 24: 641-739.

Elliot, J. A. (1923) "Cotton wilt, a seed-borne disease." *J. agric. Res.* 23: 387-393.

Ganguly, D. (1947) "Studies on the Blackburn Disease of Rice and identity of the causal organism." *J. Indian bot. Soc.* 26 (4): 233.

Henry, A. W. (1924) "Root-rots of wheat". *Tech. Bull. Minn. agric. Exp. Sta.* 22.

Johann, H. (1928) "*Penicillium* injury to corn seedlings". *Phytopath.* 18: 239-242.

Johann, H., Holbert, J. R., and Dickson, J. G. (1931) "Further studies on *Penicillium* injury to corn". *J. agric. Res.* 43: 757-790.

Kendrick, J. B. (1931) "Seed transmission and cowpea *Fusarium* wilt." *Phytopath.* 21 (10): 979-983.

Kendrick, J. B. (1934) "Seed transmission of *Fusarium* yellows of beans." *Phytopath.* 24 (10): 1159.

Kendrick, J. B. (1944) "Fruit invasion and seed carriage of tomato *Fusarium* Wilt". *Phytopath.* 34 (12): 1005.

Kulkarni, G. S. (1934) "Studies in the wilt disease of cotton in the Bombay Presidency". *Indian J. agric. Sci.* 4: 979-1048.

Leukel, R. W., and Martin, J. H. (1943) "Seed rot and seedling blight of sorghum". *Tech. Bull. U. S. Dept. Agric.* 839.

Machacek, J. E., and Greaney, F. J. (1938) "The 'Black-Point' or 'Kernel Smudge' disease of cereals." *Canad. J. Res.* C, 16: 84-113.

Machacek, J. E., and Wallace, H. A. H. (1942) "Non-sterile soil as a medium for tests of seed germination and seed-borne disease in cereals". *Canad. J. Res.* C. 20 (11): 539-557.

Mead, H. W. (1943) "Host parasite relationships in a seed-borne disease of barley caused by *Helminthosporium sativum* Pammel, King and Blake." *Rev. appl. Mycol.* 22: 60.

Mitra, M. (1935) "*Helminthosporium* disease of barley and their control". *Indian J. agric. Sci* 5: 449-484.

Muth, F. (1904) "Untersuchungen ueber die Sewankungen bei Keimkraft prufungen and ihre Uraschen". *Ber Landw. Vers. Augustenb.* 1903.

Orton, C. R. (1931) "Seed-borne parasites. A bibliography". *Bull. W. Va. Agric. Exp. Sta.* No. 245: 1-47.

Pethybridge, G. H., and Lafferty, H. A. (1918) "A disease of Flax seedling caused by species or *Colletotrichum* and transmitted by infected seeds." *Sci. Proc. Roy. Dublin Soc.* 15: 359-384.

Raistric, H. and Clark, A. B., (1919) "On the mechanism of oxalic acid formation by *Aspergillus niger*". *Biochem. J.* 13: 344.

Ray, W. W., and McLaughlin, J. H. (1942) "Isolation and infection tests with seed-and soil-borne cotton pathogen". *Phytopath.* 32: 233-238.

Robertson, D. W., Lute, A. M. and Robert Gardner, (1939) "Effect of relative humidity on viability, moisture content, and respiration of wheat, oats, and barley seeds in storage." *J. agric. Res.* 59: 281.

Rudolph, B. A., and Harrison, G. J. (1945) "The invasion of the internal structures of cotton seeds by certain Fusaria." *Phytopath.* 35: 542-548.

Simmonds, P. M., (1930) "Report of the Dominion field Laboratory of Plant Pathology, Saskatoon, Saskatchewan." In report of the Dominion Botanist for 1928, pp. 93-96, 103-105. Department of Agriculture, Ottawa.

Simpson, D. M. (1946) "The longivity of cotton seeds as effected by climate and seed treatments." *J. Amer. Soc. Agron.* 38: 32-45.

Snyder, W. C. (1932) "Seed dissemination in *Fusarium* wilt of pea" *Phytopath.* 22: 253-257.

Stakman, L. J. (1920) "*A Helminthosporium* disease of wheat and rye". *Minnesota Agr. Exp. Sta. Bull.* 191.

Subramanian, C. V. (1950) "Soil conditions and wilt disease in plants, with special reference to *Fusarium vasinfectum* on cotton". *Proc. Indian Acad. Sci.* Sect. B. 31: 67-102.

Taubenhaus, J. J., and Ezekiel, W. N. (1932) "Seed transmission of cotton wilt". *Science, N. S.* 76: 1959.

Tervet, I. W. (1945) "The influence of fungi on storage, seed viability and seedling vigour of soyabeans". *Phytopath.* 35: 3-15.

Venkat Ram, C. S. (1950) "Seed-borne fungi and loss of cotton seed viability". *J. Madras Univ. Sect.* B, XIX: 79-112.

Zachariah, A. T. (1950) "Micro-ecology of the soils of cultivated fields of South India with special reference to occurrence and physiology of *Fusarium*." Doctoral Thesis, Madras Univ.

Figs. 1-4 Photographs showing the infection of cotton seedlings by *Rhizopus Nodosus* Namyslowski in soil. Fig. 1. Showing the profuse vegetative growth and sporulation of the fungus on the cotyledons. × 2. Fig. 2. Attack of cotyledons in soil soon after emergence. × 2. Fig. 3. Ramification of the mycelium after decay of the cotyledons. × 2. Fig. 4. Shows free growth and sporulation of the fungus on soil surface. × 2½. Fig. 5. Influence on germination and vigour, of pre-soaking cotton seeds for 48 hours in spore suspension of (A) *Rhizopus nodosus* (Strain A), (B) *Aspergillus nidulans* and (C) *Aspergillus fumigatus*; extreme right is the control. 1/3 Nat. size. Fig. 6. Germination of healthy cotton seeds in sterile and non-sterile soils. Note the marked reduction in stand in the non-sterile soil. 1/8 Nat size.

A NEW SPECIES OF *SIROBASIDIUM*

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The genus *Sirobasidium* was established by Lagerheim and Patouillard in 1892. It differs from other Tremellaceous fungi in having catenulate basidia. Five species have been described (Ainsworth and Bisby, 1945) : *S. albidum* Lagerh. & Pat. and *S. sanguineum* Lagerh. & Pat. from Ecuador, *S. brefeldianum* Moeller from Brazil, *S. magnum* Boedijn from Indonesia (Netherlands Indies), and *S. cerasi* Bourdot & Galzin from France. Of these, *S. cerasi* is, according to Boedijn (1934), not a *Sirobasidium ;* indeed, he examined the type specimen of this species and even doubted whether it was a Basidiomycete (Boedijn, 1934, p. 267). Thus there are only four good species of *Sirobasidium.* So far as we are aware, no species of this genus has been recorded in India, though two species, *S. brefeldianum* and *S. magnum,* have been reported from Ceylon (Petch, 1922) and Indonesia (Boedijn, 1934) respectively. A species of this genus was collected by us from the Christian College campus, Tambaram (near Madras) in September 1951. A description of the fungus is given below.

The fungus occurs as a saprophyte on dead twigs as reddish-brown, waxy or gelatinous, shining, lobed or folded growths, 3-5 mm. in diameter and 1-1·5 mm. in height. On drying, the fungus shrinks considerably and becomes hard and black. In section, the fungus consists of loosely interwoven hyphae embedded in a gelatinous matrix. The hyphae are richly branched, hyaline, septate, 2-3 μ in diameter, with numerous clamp connections (Fig. 1, A) and anastomoses (Fig. 1, B). The basidia occur in chains and are formed centripetally. They are globose to broadly or narrowly fusiform, slightly thick-walled when old, 19 × 11 (12-32 × 8-13) μ, and divided normally by a single oblique cross-wall. Rarely there may be two oblique septa (Fig. 1, E, F). The basidia are not separated from one another by hyphal isthmi as in the case of *S. magnum* see Boedijn, 1934, p. 267, fig. 1), there being merely a distinct septum between two basidia (Fig. 1,

D-G). The basidiospores (Fig. 1, I) are sessile, one-celled, hyaline, fusoid, mostly tapering towards the base and rounded at the top, 16 × 5 (11-21 × 3-6·5) μ.

The fungus differs from species of the genus so far described. *S. albidum* and *S. brefeldianum* have been described as having white fructifications (Saccardo, 1895, 1899; Killermann, 1928). Our fungus differs from these two species in having reddish-brown fructifications. Further, it has much larger basidia and smaller basidiospores than *S. albidum;* the basidiospores are also smaller than those of *S. brefeldianum.* Our fungus, though agreeing with *S. sanguineum* and *S. magnum* in having reddish fructifications, differs from the former in not having *Tremella* — like four-celled basidia, and from the latter in not having the hyphal isthmi between basidia and in having very much smaller fructifications. Our fungus, therefore, is described as a new species.

Sirobasidium indicum sp. nov.

Acervulis rubro-brunneis, gyroso-lobatis, ceraciis vel gelatinosis, 3-5 mm. diam., 1-1·5 mm. alt.; hyphis filiformibus, valde ramosis, hyalinis, septatis, anastomosantibus; catenulis basidiorum ex 2 — 5 articulis compositis; basidiis globosis vel fusiformis, septo obliquo in duas cellulas divisis, 19 × 11 (12-32 × 8-13) μ; sporis hyalinis, continuis, fusiformibus, 16 × 5 (11-21 × 3-6·5) μ.

Hab. ad ramulos emortuos, Christian College campus, Tambaram, Madras, leg. C. V. Subramanian, 30-9-1951 (Herb. M.U.B.L. No. 540).

Fructifications reddish-brown, lobed or folded, waxy to gelatinous, 3-5 mm. in diameter, 1-1·5 mm. in height; hyphae filiform, richly branched, hyaline, septate, with numerous anastomoses and clamp connections; basidia in chains of 2-5, globose to fusiform, divided into two by an oblique septum, 19 × 11 (12-32 × 8-13) μ; spores hyaline, one-celled, fusiform, 16 × 5 (11-21 × 3-6·5) μ.

Hab. on dead twigs, Christian College campus, Tambaram, near Madras, coll. C. V. Subramanian, 30-9-1951 (Herb. M.U.B.L. No. 540).

The type specimens are deposited in the Herbarium of the Commonwealth Mycological Institute, Kew, England, and the Herbarium of the Madras University Botany Laboratory, Madras, India.

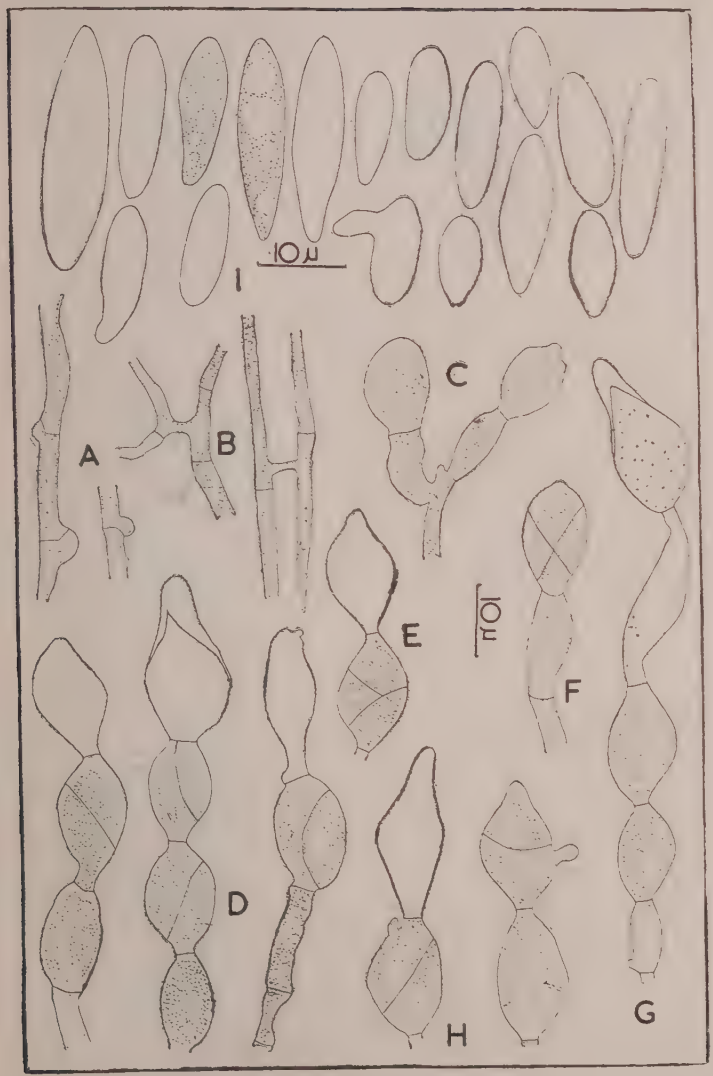

Fig. 1. *Sirobasidium indicum.* A, hyphae showing clamp connections; B, hyphae showing anastomoses; C, young basidia; D, basidia showing a single oblique septum; E, basidium divided into three by two oblique septa; F, basidium divided into four by two oblique septa; G, a chain of five basidia; the two older ones are without contents, having formed spores already; H, basidia showing formation of spores; I, basidiospores. (A—H×1000; I×1200.)

A key to the known species of *Sirobasidium* is given below, based on a study of literature :—

1. Fructifications white in colour

   2. Basidia up to 8 in a chain, basidiospores fusiform *S. albidum*

   2. Basidia 10-12 or more in a chain, basidiospores globose after they are shed *S. brefeldianum*

1. Fructifications red or reddish-brown in colour

   2. Basidium *Tremella*-like, divided by longitudinal septa into four cells *S. sanguineum*

   2. Basidium normally divided into two by an oblique septum

      3. Basidia with hyphal isthmi between them *S. magnum*

      3. Basidia not separated by hyphal isthmi *S. indicum*

We thank Professor T. S. Sadasivan, Director, University Botany Laboratory, Madras, for critically reading the manuscript.

REFERENCES

Ainsworth, G. C., and Bisby, G. R. (1945) *A Dictionary of the Fungi.* p. 293. The Imperial Mycological Institute, Kew, England.

Boedijn, K. B. (1934) The genus *Sirobasidium* in the Netherlands Indies. *Bull. Jard. bot. Buitenz.*, Ser. III, *13*: 266-68.

Killermann, S. (1928) in Engler, A., and Prantl, K., *Die natürlichen Pflanzenfamilien, 6*: 111-13.

Petch, T. (1922) Additions to Ceylon fungi II, *Ann. Roy. bot. Gard.*, Peradenya, *7*: 291.

Saccardo, P. A. (1895) *Syll. Fung., 11*: 148.

Saccardo, P. A. (1899) *Syll. Fung., 14*: 248.

ON THE LATTICE PRODUCT OF A FAMILY OF TOPOLOGIES

BY

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In this article I give generalizations (without proof) of some of Dr. Ganapathi Iyer's results contained in his papers* on the space E of integral functions. Dr. Ganapathi Iyer introduces the following topologies on E.

(a) Γ : it is the topology induced by the distance function

$$d(\alpha, \beta) = \text{U.B} \mid a_n - b_n \mid^{1/n}$$

where $\alpha = \Sigma\, a_n z^n$; $\beta = \Sigma\, b_n z^n$ are elements of E.

(b) $\Gamma(R)$: it is the topology induced by the norm

$$|| \alpha || = \Sigma \mid a_n \mid R^n, \quad (R > O)$$

He further proves that if S is a linear subset of E and $\bar{S}_{\Gamma(R)}$ the closure of S under the topology $\Gamma(R)$, then Γ is the lattice product of the topologies $\Gamma(R)$ as R varies ; further,

$$(1) \quad \bar{S}_{\Gamma} = \prod_{R} \bar{S}_{\Gamma(R)}$$

(2) $\Gamma^* = \Sigma\, \Gamma^*_{(R)}$ (i.e.) a linear functional defined over E is continuous in the topology Γ if and only if it is continuous in the topology $\Gamma(R)$ for some $R > O$.

(3) Characteristic functionals exist for every closed linear subspace S of Γ and a point α outside it.

(4) If $f(\alpha)$ is a continuous linear functional defined over a subspace S of Γ, then there exists a continuous linear functional $F(\alpha)$ defined throughout Γ such that $F(\alpha) = f(\alpha)$ for α in S. The key propositions are (1) and (2), (3) and (4) being corollories thereof.

* V. Ganapathi Iyer : The space of integral functions I, Journal Ind. Math. Society. Vol. 12, (1948).

V. Ganapathi Iyer: The space of integral functions II, Quarterly Journal of Math. (1950).

2. The purpose of this article is to draw attention to the fact that the results, (1) and (2) and hence also (3) and (4) hold for any family of "comparable" topologies, ((i.e.) given any two members of the family, one of them must be weaker than the other). The general result can be stated thus:

Let E be a vector space; $\Gamma(i)$ a family of comparable linear topologies on E for each of which properties (3) and (4) hold. Let Γ be the lattice product of $\Gamma_{(i)}$. Then we have, with the same notation as in 1.

(1)′ $\bar{S}_{\Gamma} = \prod_i \bar{S}_{\Gamma(i)}$

(2)′ $\Gamma^* = \Sigma \Gamma_{(i)}^*$

(3)′ Properties (3) and (4) mentioned above in 1 hold good. That the condition of comparability imposed on the family $\Gamma_{(i)}$ is important can be shown by constructing a family of non-comparable topologies for which result (1)′ does not hold good.

In case $\Gamma_{(i)}$ becomes a sequence of norm topologies such that the norm in $\Gamma_{(i)}$ (viz.) $|\alpha|_i$ is a monotonic increasing function of i, (and this is Ganapathi Iyer's case), we are further able to state

(1)″ Γ is metrisable with the distance function

$$d(x, y) = d(o, x - y) = \sum_i \frac{1}{2^i} \frac{||x - y||}{1 + ||x - y||}$$

(3)″ The characteristic function f, mentioned in (3) and (3)′ above can be so chosen that for all elements x.

$$|f(x)| < \frac{\delta}{2 - \delta} d(o, x)$$

where δ is the distance of the element α from the S, closed subspace S.

Another interesting case to which these results apply is had if we take the set $E_p (p > 1)$ of all sequences A: $(a_1, a_2 \ldots)$ for which $\Sigma |a_n|^{p+\varepsilon}$ converges for all $\varepsilon > o$. On E_p we can have a family of norms

$$||A||_{\varepsilon} = (\Sigma |a_n|^{p+\varepsilon})^{\frac{1}{p+\varepsilon}} \quad \varepsilon > o$$

The lattice product of all these normed topologies as ε varies can be called the l_{p+} topology. It has been shown that E_p is complete in the l_{p+} topology, though incomplete in each $l_{p+\varepsilon}$ topology.

STUDIES IN CHEMOTHERAPY, I

BY

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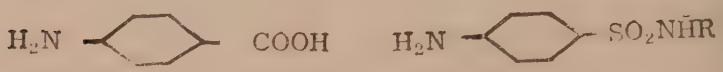
RELATION BETWEEN STRUCTURE AND ACTIVITY OF SULPHANILAMIDES

From the time that Trefouel and co-workers[1] established that p-aminobenzene sulphonamide itself was as active as Prontosil, number of derivatives of the parent substance, p-amino benzene sulphonamide have been prepared and tested for activity.[2]

Many of the derivatives were inactive and only a few had any striking activity to be incorporated into general medical use. The exact nature of the mode of action of sulphanilamides was not at all clear till about 1940. In 1940, Woods[3] and Fildes[4] established that p-aminobenzoic acid antagonised the action of the sulphonamides, on the basis of which they postulated a theory of the mechanism of action of the sulphonamide drugs — that a sulphonamide interferes with the utilisation of the substrate p-aminobenzoic acid in vital enzyme reaction by competing with p-aminobenzoic acid for its enzyme. The essential correctness of the above theory has been substantiated by many workers.[14–20] Many other theories about the relation between the structure and activity of sulphanilamides have been proposed.

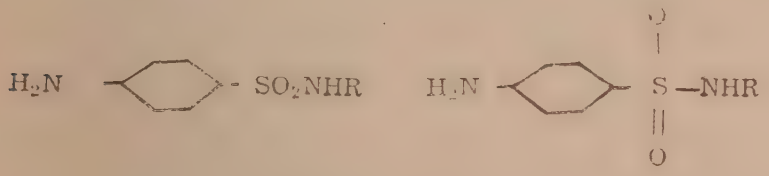


I II

Fox and Rose,[5] Schmelkes *et al*[7] and Coules[6] pointed out that there would be greater harmony if it were assumed that the acid ionic forms of sulphonamides were responsible for their activity. Bell and Roblin[8, 9] utilising the theory of Fildes proposed a theory based on the acid dissociation constants of sulphanilamides. They

stated that "the more negative the $-SO_2-$ group of an N' substituted sulphanilamide derivative, the greater is its bacteriostatic power." They make use of the acid dissociation constants of the sulphanilamide as a measure of the electro negative character of the $-SO_2-$ group. They concluded that the sulphonamide is more active in its ionised form than in the undissociated state. On the basis of their theory they were able to correlate the activity of a number of sulphonamides and predict the activity of a sulphonamide if its acid dissociation constant is known.

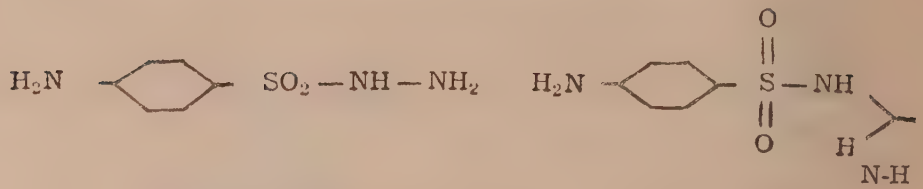
Kumler and Daniels[11] have proposed a theory for the relation between structure and activity of the sulphonamides based on their resonance structure. They point out that the fundamental factor for activity is the contribution of the reasonating form with the coplanar amino group.



III IV

This theory also comprehends the theory of Bell and Roblin because the contribution of the resonating structure also indicates the negative character of the $-SO_2-$ grouping.

On the basis of their theories Kumler and coworkers[10, 11] explain the activity of compounds which form exceptions to Bell and Roblin's theory. For example, sulphanilyl hydrazine (V) is as strong an acid as sulphanilamide but considerably less active. This is in direct contradiction to the theory of Bell and Roblin. Kumler and Daniels explain the very low activity by pointing out the existence of a hydrogen bond (VI) in sulphanilyl hydrazine which reduces the negative charge on the $-SO_2-$ group and consequently the contribution of the resonating form with the coplanar amino group is greatly reduced.

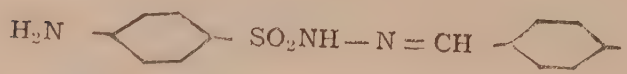


V VI

Thus according to Kumler and Daniel the presence of a strong hydrogen bond accounts for the abnormally low activity of sulphanilyl hydrazine.

If these explanations are correct, in derivatives of sulphanilyl hydrazine, where the hydrogen bond formation is prevented, the compounds should be active.

It is known that sulphanilyl hydrazine[13] reacts with benzaldehyde and acetone to give the corresponding benzylidene (VII) and acetonyl (VIII) derivatives.[12] In these derivatives there is no free hydrogen in the second nitrogen available for hydrogen bond formation.



VII

VIII

With the same idea in view the sulphanilyl derivative (X) of N'-methyl-phenyl hydrazine (IX) was prepared in the usual way. Here also as in other compounds hydrogen bond formation is impossible. These compounds were tested for their antibacterial activity.

IX X

Experimental

p-Acetaminobenzene sulphonyl chloride.

82 c.c. of freshly distilled chlorosulphonic acid was taken in a 500 c.c. roundbottomed flask provided with a mechanical stirrer.

The flask was cooled in an ice-water bath at 10°C. and 34 gms. of finely powdered acetanilide was gradually added. The addition was regulated in such a way that the temperature inside the flask did not rise above 15° C. Large volumes of hydrogen chloride were evolved. After the addition of acetanilide, the reaction mixture was heated at 60° C. for two hours to complete the reaction. After cooling, the thick liquid was poured with stirring, into 500 gms. of crushed ice. Acetaminobenzene sulphonyl chloride separated out. It was filtered by suction and washed with water. Yield, 45 gms. or 77%. M.P. of a specimen crystallised from benzene, 149° C.

Acetyl sulphanilyl hydrazine.

To 40 c.c. of 50% hydrazine hydrate solution cooled in ice was added 45 gms. of finely powdered acetamino benzene sulphonyl chloride. The mixture was stirred vigorously and cooled in ice throughout the reaction. It was kept overnight and the yellowish crystals of acetyl sulphanilyl hydrazide filtered and washed with ice cold water. Crystallised from alcohol. M.P. 178° C. Yield, 20 gms.

Sulphanilyl hydrazine.

The acetaminobenzene sulphanilyl hydrazide, 20 gms. and concentrated hydrochloric acid, 30 c.c. were mixed together and heated on a water bath, with stirring for about fifteen minutes. When the solution cleared, it was cooled and neutralised with dilute sodium hydroxide solution and kept in the ice chest overnight. The precipitated sulphanilyl hydrazide was crystallised from aqueous alcohol when it was obtained as colourless crystals. Yield, 4 gms. M.P. 132° C.

Benzylidene sulphanilyl hydrazine. VII.

One gramme of sulphanilyl hydrazide was dissolved in hot water and treated with a few drops of benzaldehyde when the benezylidene derivative separated out. It was filtered, washed with ether and crystallised from aqueous alcohol. Yield, 1 gm. M.P. 172° C.

Analysis: Calculated for $C_{13}H_{13}N_3SO_2$; S., 11·65%; N., 15·27%.
Found: S., 11·40%; N., 15·30%.

Acetonyl sulphanilyl hydrazine. VIII.

One gramme of sulphanilyl hydrazide was dissolved in just the required quantity of dilute hydrochloric acid and saturated with a few drops of acetone. The acetonyl compound that separated was filtered and recrystallised from dilute alcohol in prisms melting at 135° C. Yield, about 1 gm.

Analysis: Calculated for $C_9H_{13}N_3SO_2$; S., 16.43% ; N., 21·53%.
Found : S., 16·20% ; N., 21·30%.

Acetyl sulphanilyl N-methyl phenyl hydrazide.

2·5 gms. of methyl phenyl hydrazine was kept in 20 c.c. of pyridine and cooled to 15° C. 5·2 gms. of acetamino benzene sulphonyl chloride was added to it slowly with stirring. The solution was kept overnight at laboratory temperature. The next morning most of the pyridine was removed by distillation at reduced pressure and water added when the acetyl sulphanilyl N-methyl hydrazine separated out in needles. It was filtered, washed with water and crystallised from alcohol. Yield, 5·1 gms. M.P. 281° C.

Sulphanilyl N-methyl phenyl hydrazine. X.

4 gms. of the acetamino compound and 10 c.c. of concentrated hydrochloric acid were refluxed on a water bath for fifteen minutes when all the acetamino compound had gone into solution. After cooling, the solution was neutralised with dilute sodium hydroxide solution and kept overnight in ice chest. Sulphanilyl N-methyl phenyl hydrazide separated out as prisms. It was filtered, washed with water and crystallised from dilute alcohol. M.P. 211° C. Yield, 2·7 gms.

Analysis: Calculated for $C_{13}H_{15}N_3SO_2$; S., 11·57% ; N., 15·16%.
Found : S., 11·30% ; N., 15·02%.

The *In Vitro* Activity of the Sulphanilamides

The sulphanilamides were tested for their activity *in vitro* against a strain of *Streptococcus haemoliticus* (N.C.T.C. strain No. 1238) grown in a synthetic peptone-dextrose broth using the method of White.[21, 22]

The medium used for growing the organism as well as testing the sulphanilamides was of the following composition.

Difco peptone	..	20·0 gm.
Ordinary peptone	..	1·0 gm.
Sodium chloride	..	5·0 gm.
Phosphate buffer (pH 7·2)	..	40 c.c.
Water	..	1000 c.c.
Dextrose	..	2·0 gm.

The sulphanilamides investigated were dissolved in the glucose broth of the above composition in a concentration of 100 mgms. in 100 c.c. This stock solution was diluted in 20 c.c. test tubes with glucose broth to 10 c.c. total volume with the basal medium in a series of concentrations so that a series of 10 c.c. broth tubes contained concentrations varying from 1 in 1000 to 1 in 50,000.

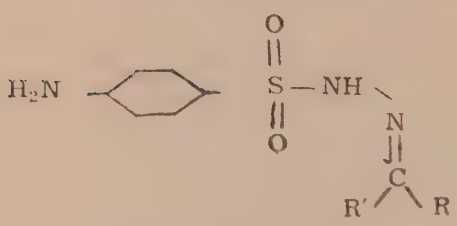
The test cultures were prepared by transfer of 1 ml. of an 18 hour broth culture to 9 ml. of sterile glucose broth and continuing the dilution to 10^{-3} of the original concentration. 1 c.c. of this test culture was added to the glucose broth of each drug dilution and incubated for 48 hours at 37° C. A control was kept with 1 c.c. of sterile broth added to 9 c.c. of glucose broth.

Only those tubes in which there was absolutely no growth were taken as having activity. The highest dilution at which there was no growth was taken as the minimum effective concentration. The results are given below.

	Name of compound.	Minimum effective concentration in mg./100 c.c.
1.	Acetyl sulphanilyl hydrazine	4,000
2.	Sulphanilyl hydrazine	4,000
3.	Benzylidene sulphanilyl hydrazine	400
4.	Acetonyl sulphanilyl hydrazine	500
5.	Acetyl sulphanilyl N-methylphenyl hydrazine	600
6.	Sulphanilyl N-methylphenyl hydrazine	300

From the table it is found that both sulphanilyl hydrazide and its acetyl derivative are not active. The compounds, 3 to 6 however show a marked activity when compared to sulphanilyl hydrazide and its acetyl derivative (1 and 2). All these compounds

have one structural feature in common, namely that the two hydrogen atoms in the hydrazine part of the sulphanilamide are not available for hydrogen bond formation either due to removal by condensation with carbonyl compound, benzaldehyde (3) or acetone (4) or due to substitution (5 and 6) as shown below.



VII R = H, R′ = 

VIII R = R′ = Me

Since in all other respects they resemble the parent sulphanilyl hydrazide which is inactive, the activity must be due to this prevention of hydrogen bond formation thus proving that Kumler and Daniels' suggestion that sulphanilyl hydrazide is inactive by virtue of hydrogen bond formation is essentially correct. It can also be seen that the parent sulphanilamide (6) possesses a greater activity than its acetyl derivative (5).

ACKNOWLEDGEMENTS

My grateful thanks are due to Prof. K. N. Menon and Prof. V. Subramanian for their kind interest in the work and to the Trustees of the Lady Tata Memorial Trust for the award of a Lady Tata Memorial Scholarship.

REFERENCES

1. Trefouel, Trefouel, Nitt and Bovett, *C. R. Soc. Biol. Paris*, 1935, *120*, 756.
2. E. H. Northey, *"Sulphonamides and Allied Compounds"*, A. C. S. Monograph No. 106, Reinhold Publishing Corporation, First Edition, New York, 1948.
3. Woods, *Brit. J. exp. Path.*, 1940, *21*, 74.
4. Fildes, *Lancet*, 1940, (238), 955.
5. Fox and Rose, *Proc. Soc. exp. Biol., N. Y.*, 1942, *50*, 142.
6. Cowles, *Yale J. Biol. Med.*, 1942, *14*, 599.
7. Schmelkes, Wyss, Marks, Ludwig and Strandskov., *Proc. Soc. exp. Biol., N. Y.*, 1942, *50*, 145.

8. Bell and Roblin, *J. Amer. chem. Soc.*, 1942, *64*, 2905.
9. Bell and Roblin, *Ann. N. Y. Acad. Sci.*, 1943, *44*, 449.
10. Kumler and Halverstadt, *J. Amer. chem. Soc.*, 1941, *63*, 2182..
11. Kumler and Daniels, *J. Amer. chem. Soc.*, 1943, *65*, 2190.
12. Curtius and Stoll, *J. prakt. Chem.*, 1926, *112*, 125.
13. Mann and Holliman, *J. chem. Soc.*, 1942, 738.
14. McIllwain, *Brit. J. exp. Path.*, 1940, *21*, 136.
15. McIllwain, *Ibid.*, 1942, *23*, 95.
16. McIllwain and Hawking, *Lancet*, 1943, *1*, 449.
17. Erlenmeyer et al, *Helv. chim, Acta*, 1942, *25*, 1066.
18. Miller et al, *Ber. dtsch. chem. Ges.*, 1942, *75B*, 1108.
19. Kuhn, Miller and Wieland, *Ber. dtsch. chem. Ges.*, 1941, *74B*, 1605.
20. Nitti and Matti, *C. R. Soc. Biol. Paris.*, 1942, *136*, 402.
21. White, *J. Bact.*, 1939, *38*, 549.
22. White, *Proc. Soc. exp. Biol., N. Y.*, 1940, *43*, 2135.

PHYSIOLOGICAL ZOOLOGY AND FISHERIES

BY

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Speaking in general terms, the main objective of fishery biology is to increase the available fishery resources by the exploration of new fields and facts concerning the organisms that comprise the fisheries. Their periodical abundance or scarcity, habits, movements and life histories, if fully known, would help us to take full advantage of what already exists in Nature, and to work out measures to prevent their depletion or even total extermination by indiscriminate fishing. In this aspect of work, we have only made a beginning in India and have yet to learn a great deal, considering the vast number of species that live in our waters and the wide variety of habitats they confront.

An equally important aspect of fishery development is to attempt to increase the already existing resources. If we know well enough about the constitution and habits of our fishes, can we increase their populations by natural or artificial means or by planned cultivation ? It is in this direction that physiological zoology can contribute to fisheries development by providing the basic data on conditions of life and adaptation of culturable fishes on which the success of pisciculture depends. I shall now indicate a few aspects of functional activities of organisms that may help us solve some of our problems.

1. *Temperature :* We are indebted to the work done by European workers in countries bordering the North Atlantic for much of our fundamental knowledge of the science of marine fisheries. Problems of fresh water fisheries have also until very recently, received close and critical study only in Europe and America. While applying this most useful information to fishery problems in the tropics we have to contend with the considerable difference in the temperature conditions that prevail between European waters and our own. In many tropical regions there is the complete absence of sharply demarcated periods of winter and summer with the result that annual fluctuations in temperature of tropical waters

are small as compared with those of the colder regions. The natural inland waters such as lakes are perpetually active in the biological sense, that is, without any period of dormancy which, if present at all, is the period of total drying up of the smaller areas of water in summer resulting in the destruction of most of the organisms contained therein.

It is well known that biological and chemical processes are activated by rise in temperature. Consequently the rate of growth in tropical waters is much faster than in colder regions; often we may have within the same year several generations of a species whose opposite number in temperate waters may require one full year or more to pass from one period of spawning to the other. While the rapid growth and early attainment of sexual maturity are an advantage in the culture of tropical fish, the small size of some of the quick growing species is a disadvantage as they seldom grow to the same size as the slow-growing ones. In the selection of an ideal species for culture one has to strike an advantageous mean between the mass of food produced and the time involved in producing it. In successful large scale operations it is usual to select a species which is tolerant to variations in temperature and adjusts its behaviour to suit widely varying temperatures.

2. *Salinity and Regulation*: Marine fishes of the coast tolerate slight variations in salinity corresponding to the seasonal changes taking place in the seas but there are not many marine species of commercial value which can survive great changes in salinity. Migratory forms like the *Hilsa* are known to live in fresh and salt water. Marine teleosts or bony fishes have highly developed powers of adjusting their salt and water content—osmoregulations as it is technically spoken of—involving steady elimination of salts through the gills to maintain a concentration much below that of the sea water, but the power of penetration of such fishes into fresh water is confined only to a few groups. It is essential for us to know which species have high powers of regulating their salt and water content, as these would be the most suitable for culture work as shown below. The milk fish, *Chanos chanos,* and many mullets are capable of living in waters of varying salt content, especially in their young and juvenile stages, and have been cultivated for centuries in S. East Asian and other countries. They seem to offer new fields for further development in our own country, if we could determine the most suitable for farming and the most suitable habitats for successful culture. Methods introduced to cultivate *Chanos* in India have already yielded valuable results.

The long coast line of India is indented by large and small rivers flowing into the sea, forming deltas, and a number of smaller creeks and backwaters. The very considerable amounts of fresh water brought down by rivers in spate during the monsoons contribute to the formation of a significant habitat on the coast line which may be called "the blackish water zone". The marine lagoons, so characteristic of the Coromandel Coast of India form a distinct zone of great potentiality for piscicultural operations which could be developed like the "tambaks" of Indonesia. These areas, as indicated by surveys carried out in the Gangetic delta, the Chilka Lake, the Madras backwaters etc., have a high biological productivity as shown by their rich fauna both in numbers and species. Many young fishes and prawns from the sea are known to ascend into the habitats described above to feed and grow, only to return to the sea when full-grown where alone they breed.

Although in cultural operations, the considerable variations in salinity occurring in the coastal zones are a hindrance to their full utilisation, a fuller knowledge of the physiology of the species would help to obviate certain difficulties. The predominant elements of the fauna and flora of these zones are known, and judged by the ecological data available there are species of Cichlids, Mullets and Perches among fishes and many species of Penaeid and Palaemonid prawns among shell-fish that could probably be employed for culture work. I venture to think that more intensive studies on the adaptational behaviour of these estuarine animals, with a view to selecting such of those as may be suitable for artificial culture would yield valuable results than direct observations on these animals in the field which may take many years. With some experimental data gathered it should be easy to apply them to practical field tests.

3. *Importance of trace elements in growth :* A line of work which has come to the fore recently is that which concerns the importance of minute traces of certain elements for the growth of plant life as well as for the continued vigour and growth of certain animals. It is well-known that minute plant life, particularly the flagellates and the diatoms, form the first and the strongest link in the chain of marine and fresh water life. More than 25 years ago Allen found that it was possible to culture artificially certain marine diatoms only either in sea water or in artificial sea water to which a small amount of natural sea water has been added. The presence of the natural sea water seemed to act somewhat like vitamins in food substances ! A very effective method of artificial

culture of diatoms and zooplanktonic organisms has been developed by Gross which has been successfully employed on a large scale in what are called plankton shafts. In this method, an extract of the earth or the soil (hence called in German "Erdschreiber" method) is added to the culture, in addition to inorganic salts, presumably to provide the trace substances essential for growth, of which, it has been found by Harvey that manganese was one. The importance of phosphates, nitrates and silicates in plankton production and control is too well known to need emphasis.

The problem of finding the essential nutrient salts for culturing organisms, although far from being fully solved, has helped us to appreciate the importance of fertilizers in increasing the productivity of natural waters. The subject is of particular interest to our country with a large number of small areas of inland waters both saline and fresh water suitable for artificial treatment. There is great need and urgency for fundamental research on the lines indicated above. Recently, British biologists led by Gross achieved striking results in their experiments on a Scottish Loch (an arm of the sea) where they grew flat-fish under what may be called artificially manured conditions by adding considerable amounts of nutrient salts. The detailed results now published may well provide the starting point for a new phase in pisciculture. The use of chemical fertilizers for increased yields in fisheries may not necessarily be economical or practicable even in industrially advanced countries like the U. K. Under the present economic conditions in India any extensive use of chemical fertilizers for culturable water masses appears remote. The fundamental idea that waters, like agricultural land, can be fertilized to increase the yields should, however, receive wide publicity in this country, so that we could explore possibilities of utilizing organic and other waste products for enriching our fallow waters.

A better appreciation of the properties of the natural waters would again be of great value. The presence of small amounts of chloride is essential for the physiological regulation of most freshwater animals. Owing to the widespread occurrence of the phenomenon of active ion-absorption, most aquatic animals are able to utilize these irons, even when they are present in the surroundings in exceedingly minute quantities. Secondly, calcium is essential for growth and for the maintenance of healthy epithelia through its influence on membranes, thereby playing a vital part in adaptation to the surroundings. Chloride and calcium ions present in water are thus important factors to be considered in fish cultural opera-

tions as also the acidity or alkalinity of the waters. It is often found that acid waters invariably contain only lean fish not growing to any appreciable size which are obviously uneconomical. Fortunately, acid waters are few in India. Fish culturists always find it essential to correlate the properties of the environment with the physiology of the edible fishes cultivated.

4. *Reproduction :* The physiology of reproduction of species which are of value in fish culture is a problem which requires special attention. Most of such species do not spawn in captivity and hence the elaborate technique of collecting the fish spawn or fry from the natural spawning habits and transporting them to fish farms has had to be developed. If methods are found to induce adult fish to produce young ones in captivity the fish cultural procedures would be substantially simplified.

A few aspects of physiological work relating to fisheries have been indicated here but only the fringe of a vast field has been touched upon. Active investigations on problems of growth, adaptation, and reproduction are essential and should commend themselves to our rising zoologists who will find in them not only topics of absorbing scientific interest but of successful practical application to fisheries.

THE LIBERATION OF TRYPTOPHAN DURING ENZYMIC PROTEOLYSIS

BY

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INTRODUCTION

During the isolation of tryptophan from the pancreatic digest of Casein, Hopkins and Cole[1] observed that the red colour with bromine water characteristic of 'free' tryptophan reached its maximum only at the end of several days of hydrolysis. Abderhalden and Reinbold[2] found that edestin digested with pancreatin for four days, had its tryptophan moiety in a soluble and dialysable form. Fürth and Lieben,[3] successively digested blood fibrin with pepsin, trypsin, and erepsin and found that even after continuing the digestion for a period of two weeks, only 60% of the tryptophan was in the free condition and concluded that there was no justification for assigning to tryptophan a particularly exposed position in the protein molecule and that the liberation was only gradual, proceeding largely in parallel with the splitting of peptide groups. Moreover, they used a phosphotungstic acid treated digest for the determination of the free amino acid, which as shown by the subsequent work of Vanslyke[4] *et al* could form a highly insoluble phosphotungstate.

Ragins[5] studied the rate of liberation of tryptophan from Witte's peptone, casein, edestin, and squash seed globulin during pancreatic digestion and then during successive digestion with pepsin, trypsin and erepsin. She assumed the mercuric sulphate precipitable tryptophan in the free condition but found it difficult to explain the formation of a complex mercury precipitate in her peptic digests, which as was evident from the subsequent work of Onslow[6] could be only due to tryptophan peptides. In the case of Witte's peptone, she found rather curiously that one third of the total potential tryptophan was free even before incubation with

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trypsin, and at the end of one hour an additional one third was liberated. In the first hour, 75% of the total available tryptophan was liberated in the case of casein, a little less than 50% from edestin, and 40% from squash seed globulin. She concluded that trypsin acting after pepsin did not increase the rate of liberation and the pancreatic enzymes alone completely liberated the tryptophan. She attributed the difference in the rate of liberation of tryptophan between the three proteins as due to their difference in solubility in the hydrolysis medium, rather than to a difference in the mode of attachment of the amino acid in the molecule.

The liberation of free amino acids during peptic hydrolysis has been a controversial question.[6] Even though the formation of small quantities of free amino acids notably tyrosine, during prolonged peptic hydrolysis had been noticed for a long time, Northrop,[7] Calvery,[8] it was Bergmann[9] who demonstrated beyond doubt, the peptidase activity of pepsin and papain by synthesising suitable substrates for their action. Abderhalden[10] reported the formation of 'free' tryptophan during peptic hydrolysis by employing the highly specific bromine colour reaction for its identification. Even though the specificity of the test for 'free' tryptophan has been placed beyond doubt by the work of Neuberg [11] Abderhalden,[12] and Fearon,[13] the optimum conditions for carrying out the test and the interfering factors on the colour were not well investigated previously. It was observed during the present investigation that the colour formation was easily effected in an acetate buffer medium of pH 3·8, by 0·1 N bromine water and the colour could be brought into solution in 60% acetic acid for comparison. Among the other halogen reacting amino acids it was found that the colour intensity due to 1 mg. of tryptophan was nearly doubled by the presence in the medium of 0·8 mg. Tyrosine, 0·4 mg. Cystine, and 0·2 mg. Histidine although by themselves these amino acids do not give any colour with bromine water. The report of Abderhalden[10] that his neutralized peptic digest of casein gave a strong bromine colour after the addition of a speck of tryptophan could quite possibly be explained as due to the intensification of the added tryptophan colour by the tyrosine present in the digest which has previously been shown to be liberated during peptic hydrolysis.[7, 8] However, this does not clearly preclude the possibility of tryptophan being set free during peptic hydrolysis.

Recently, Elvehjem[14] and co-workers have reported that almost the whole of the tryptophan from casein, wheat gluten and fibrin could be liberated in 24 hours, when their hydrolysis was conduct-

ed with a high concentration of pancreation and erepsin coupled with vigorous shaking of the reaction vessel. However, they have used the micro biological method for the assay of the free amino acid, the specificity of which is not certain, and no measurement is made of the extent of hydrolysis.

Materials

Casein used in these experiments was prepared from fresh milk by the method of Cohn and Hendry[16] as given in Clarke's organic synthesis. Edestin and beef blood fibrin were prepared by standard methods. Erepsin solution and papain for digestion were prepared in the laboratory, according to Damodaran and Anantanarayanan.[17] The enzymic digestions were carried out according to Damodaran and Krishnaswamy.[18] Suitable controls were run simultaneously along with the experimental digestions. The tryptophan content of pepsin, trypsin and papain was determined by the method of Bates[19] and the values were allowed for in the digests.

Pepsin contained	..	0·2%	tryptophan
Trypsin „	..	1·6%	„
Papain „	..	1·0%	„

Present Investigation

A study has been made of the liberation of tryptophan during the successive enzymic digestion of the three proteins, casein, fibrin and edestin with pepsin, trypsin, and erepsin. Digestion of the three proteins, by activated papain was also carried out. The extent of hydrolysis was followed during the course of digestion. Tryptophan determinations in the digest were carried out according to the paradimethyl amino benzaldehyde method of Horn and Jones[15] and by the bromine method developed in the course of this investigation.

Estimation of Apparent Tryptophan

2·0 to 4·0 ml of the neutralized digest was made upto 10·0 ml and suitable aliquots depending on the trytophan content were used for the determination by the aldehyde method.

Detection of 'free' Tryptophan in the Digest

10·0 ml of the peptic digest was pipetted out into a 50 ml. conical flask and 2 mg. tyrosine, 1 mg. cystine, and 0·5 mg. histi-

dine were added to it from a stock solution of the same composition. The pH was adjusted to 3·8 by the addition of normal sodium acetate and dilute bromine water was added gradually with shaking. The formation of a pink colour would indicate the presence of 'free' tryptophan. The peptic digests of casein and edestin gave no colour while a slight colour developed in the case of fibrin.

Estimation of 'free' Tryptophan

Reagents:—(i) Standard tryptophan solution containing 1 mg./ml. was prepared by dissolving 50 mg. L tryptophan in the minimum amount of 0·1 N hydrochloric acid and the volume made up to 50·0 ml.

(ii) *Synthetic amino acid mixture*:—100 mg. L cystine and 25 mg. L histidine monohydrochloride were dissolved in 50 ml. 0·5 N sulphuric acid.

(iii) *Sodium metabisulphite solution* 0·2 M:—3·8 gm. of the pure salt was dissolved in 100 ml. distilled water.

(iv) *Bromine water.* 25·0 ml. saturated bromine water were diluted to 100 ml.

(v) *Acetate Buffer* 8·8 ml. of N acetic acid were mixed with 1·2 ml. N sodium acetate.

(vi) *Glacial acetic acid.* A. R. quality was used.

Procedure. 5·0 ml. of the peptic digest of fibrin was pipetted out into into a 50 ml. conical flask and 0·5 ml. of the amino acid mixture solution was added to this. 0·5 ml. N sodium acetate was added to adjust the pH to 3·8 and 1·0 ml. of the acetate buffer was also added. The bromine water was added from a micro burette drop by drop, with shaking till the colour intensity became maximum. 0·5 ml. of the sodium metabisulphite solution was added to the mixture with shaking to destroy the excess free bromine present. The contents were centrifuged for five minutes at 2000 r.p.m. and the supernatant was decanted off into the original flask. The coloured residue was extracted twice, with 5 and 2 ml. portions of acetic acid, centrifuged, and the centrifugate transferred to the original flask. Matching was done with a standard developed under the same conditions using 0·2 to 0·5 mg. tryptophan. There was proportionality between colour and concentration over the range 0·5 to 4·0 mg. tryptophan in a total volume of 30 ml. and in this case the final volume was made up to 15 ml. as the amount of tryptophan present was small.

Estimation of 'free' Tryptophan in the tryptic, ereptic, and papain digests

(Reagents same as above.) 5·0 to 10·0 ml. of the digest depending on the tryptophan content was neutralized to pH 3·8. The synthetic amino-acid mixture was not added as the digests already contained sufficient amounts of these to intensify the colour. 2·0 ml. of the acetate buffer was added and saturated bromine water diluted with an equal volume of water was used for colour development. 15·0 ml. of acetic acid was used for the extraction of the colour. A suitable standard was developed under the same conditions but with the addition of the synthetic amino acid mixture. Comparison was made as above.

Extent of Hydrolysis

For calculating the extent of peptide hydrolysis aminonitrogen determined according to Sorensen was compared with the amino-nitrogen on complete hydrolysis, the latter value bing calculated from the N distribution values given in Plimmer.[20] The tryptophan values and the peptide hydrolysis values were corrected after making due allowance for the controls. The apparent tryptophan values by the aldehyde method in the case of edestin and fibrin were calculated on the basis of the values obtained by alkaline hydrolysis. In the case of casein, as the free tryptophan in the digest rose to 1·45% and the value obtained on alkaline hydrolysis was only 1·30%, the percentage split was not calculated and the values were given as such.

To ascertain whether any tryptophan was destroyed during peptic digestion, aliquots of 10·0 ml. were hydrolysed with 28% barium hydroxide in the autoclave and the tryptophan was estimated in the hydrolysate. It was found that there was no destruction of tryptophan during peptic digestion as could be seen from the following table.

Protein	Tryptophan per cent	
	Before digestion	After digestion
Fibrin	3·80	3·92
Casein	1·30	1·30
Edestin	1·40	1·58

Results and Discussion

Regarding the liberation of tryptophan during peptic hydrolysis of the three portions, the bromine water test remained negative in the case of casein and edestin from the beginning, to the day when the digests were put on trypsin. There was a faint colour given by fibrin on the addition of a large volume of bromine water. Estimates made on the 4th day digest revealed that 6% of the total tryptophan of fibrin was in the free condition. As the accuracy of the method itself is about 5%, not much reliance could be placed on this value. Hence, with the commercial enzyme used in these digestions it can be concluded that no tryptophan was set free during peptic digestion.

It is well known that the aldehyde reactions are answered by both the free and the combined tryptophan. While working with the p-dimethylaminobenzaldehyde method of estimating tryptophan, Horn and Jones[15] have concluded that maximum colour formation with free tryptophan takes place only at room temperature, while for that in combination, takes place both at room temperature and at 55°C. They also conclude that tryptophan in combination produces more colour with the aldehyde reagent than an equivalent amount of the free acid. Using the glyoxylic acid method, Shaw and McFarlane,[21] have also concluded that tryptophan in combination has more chromogenic power than the free acid. The values obtained by the aldehyde method in all the peptic digests and during the early stages of tryptic digestion are in accordance with this view.

Taking the individual proteins, in the case of the peptic digest of casein the peptide hydrolysis reached a value of 20·43% in a week when the apparent tryptophan by the aldehyde method increased from 1·14% at the end of one hour to 1·83% on the 2nd day and decreased to 1·7% on the 8th day. In the same manner peptide hydrolysis reached a value of 15·3% in 7 days in the case of edestin, while the apparent tryptophan increased from 92·85% to 124·3%. In the case of fibrin the peptide hydrolysis reached a value of 17·1% in 7 days and the apparent tryptophan increased from 97·36% at the end of the first hour to 134·2% on the 6th day and decreased to 118·4% on the 7th day. The maximum peptide hydrolysis achieved in peptic digestion was only about 20% and since there was no free tryptophan the high apparent tryptophan values could be quite possibly attributed to the presence of highly chromogenic polypeptides.

In the case of the tryptic digest of casein the apparent tryptophan value rose from 1·42% at the end of one hour to 1·5% at the end of 5 hours and had a sudden fall to 1·17% on the first day from which the value gradually rose to 1·4% on the 10th day and remained stationary till the 20th day. The 'free' tryptophan value represented by the bromine method was 0·98% at the end of 2 hours and rose gradually to 1·40% on the 3rd day and after a slight fall on the 8th, reached the maximum of 1·45% on the 15th and remained at that till the 20th. The rapid fall in the aldehyde value in the tryptic digestion could be attributed to the rapid splitting off of tryptophan from the poly peptides resulting in a corresponding decrease in the chromogenic power. The values for the apparent tryptophan from the first day of the tryptic digestion till the 20th was lower than the free tryptophan indicating a high concentration of free tryptophan in contrast to peptides in the digest. When the 12th day tryptic digest was put on erepsin, the initial apparent tryptophan value of 1·41% decreased to 1·30% on the 2nd day and remained stationary till the 7th. On the other hand, the value for free tryptophan which was initially at 1·45% decreased to 1·40% on the 2nd day, then 1·37% on the 4th and continued at that till the 7th day. The only conclusion that could be drawn from these values is that trypsin followed by erepsin did not lead to any further liberation of tryptophan. The extent of peptide hydrolysis and the percentage of free and apparent tryptophan liberation with time are represented in tables 1 to 3.

In the case of the tryptic digest of edestin the apparent tryptophan value rose to a maximum of 140·1% at the end of 2 hours, after which it gradually decreased to a value of 91·43% on the 18th day. The apparent tryptophan value in the tryptic digest was higher than in peptic and the chromophoric substance appeared to be only gradually destroyed by trypsin. The reason for this abnormally high value could only possibly be attributed to the presence of complex chromogenic peptides, which lost this property on further hydrolysis. The 'free' tryptophan value in the case of edestin was 74·29% at the end of 6 hours and it gradually rose to 95·01% on the 18th day, when the apparent tryptophan value was 91·43%. When a 10th day tryptic digest was put on erepsin, the apparent tryptophan value rose to 95·72% on the second day, 105·7% on the 4th day and finally reached 114·3% on the 7th day. The 'free' tryptophan value was 100 at the start and continued at that till the end of the 7th day. This

TABLE I

CASEIN PEPTIC DIGEST

5 ml. of digest contains 93·10 mg. protein.

5 ml. of digest contains 10·27 mg. amino nitrogen = 6·90 ml. alkali.

Total nitrogen present = 16·19% ; Moisture content = 8·20%.

Period of digestion.	Formol titre. ml.	Increase in titre. ml.	Peptide split. %	Tryptophan liberated per cent. Aldehyde Method.
0·0	0·40	0·0	—	—
1 hr.	0·66	0·26	3·8	1·14
2 hr.	1·02	0·62	9·0	1·20
3 hr.	1·15	0·75	10·9	1·30
4 hr.	1·17	0·77	11·2	1·47
5 hr.	1·22	0·82	11·9	1·50
6 hr.	1·26	0·86	12·5	1·68
1st day	1·38	0·98	14·2	1·68
2nd day	1·61	1·21	17·5	1·83
3rd day	1·62	1·22	17·7	1·73
4th day	1·70	1·30	18·8	1·73
5th day	—	—	—	1·73
6th day	1·81	1·41	20·43	1·70
7th day	1·81	1·41	20·43	1·69
8th day	—	—	—	1·70

TABLE II

CASEIN TRYPTIC DIGEST

5 ml. of digest contains 79·8 mg. protein.
5 ml. of digest contains 8·80 mg. amino nitrogen = 5·914 ml. alkali.

Period of digestion.	Formol titre. ml.	Peptide split. %	Tryptophan liberated per cent.	
			Aldehyde method.	Bromine method.
10 min.	1·84	31·1	—	—
1 hr.	2·04	34·5	1·42	—
2 hr.	2·15	36·3	1·44	0·98
3 hr.	2·26	38·2	1·50	1·05
4 hr.	2·32	39·2	1·50	—
5 hr.	2·40	40·6	1·47	—
6 hr.	2·41	40·7	1·50	—
1st day	2·71	45·8	1·17	1·28
2nd day	2·87	48·5	1·23	1·32
3rd day	2·95	49·9	1·23	1·40
5th day	3·06	51·7	1·30	1·40
7th day	3·33	56·3	1·30	—
8th day	—	—	1·30	1·35
10th day	—	—	1·40	1·35
15th day	3·48	58·80	1·40	1·45
20th day	3·60	60·87	1·40	1·45

TABLE III

CASEIN EREPTIC DIGEST

5 ml. of digest contains 66·5 mg. protein.
5 ml. of digest contains 7·3 mg. amino nitrogen = 4·93 ml. of alkali.
12th day Tryptic Digest put on Erepsin.

Period of digestion.	Formol titre. ml.	Peptide split. %	Tryptophan liberated per cent.	
			Aldehyde method.	Bromine method.
0·0	3·21	65·1	1·41	1·45
2nd day	3·36	68·2	1·30	1·40
4th day	3·55	72·2	1·30	1·37
7th day	3·61	73·2	1·30	1·37

abnormality could be explained only thus. The tryptophan content of 1·4% for edestin obtained on alkaline hydrolysis, might be a lower value than the actual tryptophan content, as it is almost impossible to carry out alkaline hydrolysis without some destruction of tryptophan. The rate of increase of tryptic hydrolysis after the 10th day became so slow that the rate of splitting of tryptophan might have slowed down. On such a digest erepsin might act and liberate more tryptophan which might be in fact the total tryptophan available from edestin and consequently the value 1·6 could be taken to represent its real tryptophan content. The extent of peptide hydrolysis and the percentage of free and apparent tryptophan liberation with time for edestin are represented in Tables 4 to 6.

TABLE IV

EDESTIN PEPTIC DIGEST

5 ml. of digest contains 86·35 mg. protein.

5 ml. of digest contains 9·64 mg. amino nitrogen = 6·47 ml. alkali.

Tryptophan value by alkaline hydrolysis = 1·4%.

Total nitrogen = 18·60% Moisture = 6·0%

Period of digestion.	Formol titre. ml.	Increase in titre. ml.	Peptide split. %	Tryptophan liberated per cent.	Hydrolysis per cent based on alkaline hydrolysis value.
				Aldehyde method.	Aldehyde method.
0·0	0·46	—	—	1·30	92·85
1 hr.	0·57	0·11	1·7	1·42	101·40
2 hr.	0·63	0·17	2·6	1·47	105·0
5 hr.	0·82	0·36	5·6	1·47	105·0
1st day	1·05	0·59	9·1	1·53	109·2
2nd day	1·07	0·61	9·4	1·54	110·0
3rd day	1·23	0·77	11·9	1·62	115·7
4th day	1·33	0·87	13·4	1·63	116·4
5th day	—	—	—	1·72	122·8
6th day	1·38	0·92	14·2	1·74	124·3
7th day	1·45	0·99	15·3	1·74	124·3

TABLE V

EDESTIN TRYPTIC DIGEST

5 ml. of digest contains 74·0 mg. protein.
5 ml. of digest contains 8·26 mg. amino nitrogen = 5·55 ml. alkali.

Period of digestion.	Formol titre. ml.	Peptide split. %	Tryptophan liberated per cent.		Hydrolysis per cent based on alkaline hydrolysis value.	
			Aldehyde method.	Bromine method.	Aldehyde method.	Bromine method.
10 min.	1·94	34·4	1·80	—	128·6	—
1 hr.	1·95	35·1	1·80	—	128·6	—
2 hr.	2·04	36·8	1·96	—	140·1	—
3 hr.	—	—	1·91	—	136·4	—
4 hr.	2·05	37·0	1·91	—	136·4	—
5 hr.	2·10	37·8	1·87	—	133·6	—
6 hr.	2·18	39·3	1·69	1·04	120·7	74·29
1st day	2·63	47·4	1·57	—	112·1	—
3rd day	2·99	53·9	1·50	—	107·2	—
4th day	—	—	—	1·10	—	78·57
5th day	3·28	59·1	1·45	1·12	103·6	80·00
6th day	—	—	1·40	1·20	100·0	85·72
13th day	—	—	—	1·30	—	92·85
14th day	3·75	67·6	1·35	—	96·42	—
18th day	3·89	70·1	1·28	1·33	91·43	95·01

TABLE VI

EDESTIN EREPTIC DIGEST

5 ml. of digest contains 61·65 mg. protein.
5 m. digest contains 6·90 mg. amino nitrogen = 4·62 ml. alkali.
10th day Tryptic Digest put on Erepsin.

Period of digestion.	Formol titre. ml.	Peptide split. %	Tryptophan liberated per cent.		Hydrolysis per cent based on alkaline hydrolysis value.	
			Aldehyde method.	Bromine method.	Aldehyde method.	Bromine method.
0·0	3·52	76·1	1·33	1·42	—	101·4
2nd day	3·73	80·7	1·34	1·40	95·72	100·0
4th day	3·86	83·5	1·48	1·40	105·7	100·0
7th day	3·97	85·9	1·60	1·40	114·3	100·0

In the tryptic digest of blood fibrin the apparent tryptophan value which was 118·4% at the end of peptic digestion, rose to 131·6% at the end of one hour, to 134·2% at the end of 3·5 hours, after which it remained stationary at 131·6% till the end of the first day. The value began to fall gradually and finally reached 73·69% on the 21st day. The free tryptophan was 44·73% on the second day and rose to a maximum of 76·3% on the sixteenth day. This value agreed with the apparent tryptophan value of the same interval, and the latter value too could be representing more or less free tryptophan. In this case, the hydrolysis of the highly chromogenic polypeptide complex proceeded rather more slowly than in the other two cases. When a 13-day old tryptic digest was put on erepsin, the apparent tryptophan value which was 78·9% initially, rose to 84·2% on the 2nd day, 95·26% on the 4th day and finally came to 96·32% on the 7th day. The free tryptophan value by the bromine method was 86·84% at the beginning

TABLE VII

FIBRIN PEPTIC DIGEST

The strength of Formol Alkali is 1·063 N/10 ≡ 1·488 mg. N. per ml.
5 ml. digest contains 84·45 mg. protein.
5 ml. digest contains 10·51 mg. amino nitrogen = 7·06 ml. alkali.
Tryptophan content by alkaline hydrolysis =3·8%.
Total nitrogen = 15·75% ; Moisture = 15·18%.

Period of digestion.	Formol titre. ml.	Increase in titre. ml.	Peptide split. %	Tryptophan liberated per cent.	Hydrolysis per cent based on alkaline hydrolysis value.
				Aldehyde method.	Aldehyde method.
0·0	0·36	0·0	—	—	—
1 hr.	0·84	0·48	6·8	3·70	97·36
2 hr.	0·95	0·59	8·3	3·90	102·70
3 hr.	0·96	0·60	8·5	3·92	103·10
4 hr.	1·80	0·72	10·2	3·95	104·0
5 hr.	1·09	0·73	10·3	3·95	104·0
6 hr.	1·15	0·79	11·2	3·96	104·2
1st day	1·20	0·84	11·9	4·00	105·3
2nd day	1·36	1·00	14·2	4·20	110·5
3rd day	1·39	1·03	14·6	4·20	110·5
4th day	1·49	1·13	16·0	4·30	113·2
5th day	—	—	—	4·30	113·2
6th day	1·53	1·17	16·6	5·10	134·2
7th day	1·57	1·21	17·1	4·50	118·4

and satisfactory determinations could not be carried out on the subsequent days because of strong interfering tints. The rise in the apparent tryptophan value in this case was well within the total tryptophan value obtained on alkaline hydrolysis. In the case of the tryptic digest the relative rise in the free tryptophan value after the 8th day to the 16th was only 2·61% indicating that the action of trypsin virtually stopped at that period without any further appreciable liberation of tryptophan. The increase in the peptide split also was small. Erepsin could be expected to hydrolyse this digest vigorously and the increase in the apparent tryptophan value to 96·32% could in fact be the free tryptophan liberated by erepsin. In this case the liberation took place gradually and in accordance with the splitting of the peptide group. The extent of peptide hydrolysis and the percentage of free and apparent tryptophan liberation with time for fibrin are represented in Tables 7 to 9.

TABLE VIII

FIBRIN TRYPTIC DIGEST

5 ml. of digest contains 72·4 mg. protein.

5 ml. of digest contains 9·0 mg. amino nitrogen = 6·05 ml. alkali.

Period of digestion.	Formol titre. ml.	Peptide split. %	Tryptophan liberated per cent.		Hydrolysis per cent based on alkaline hydrolysis value.	
			Aldehyde method.	Bromine method.	Aldehyde method.	Bromine method.
10 min.	1·52	25·1	—	—	—	—
1 hr.	1·83	30·2	5·0	—	131·6	—
2 hr.	1·84	30·4	5·0	—	131·6	—
3½ hr.	1·83	31·9	5·1	—	134·2	—
4½ hr.	2·04	33·7	5·0	—	131·6	—
6 hr.	2·16	35·7	5·0	—	131·6	—
1st day	2·61	43·1	5·0	1·60	131·6	42·1
2nd day	2·91	48·1	4·4	1·70	115·8	44·73
3rd day	3·04	50·2	4·0	—	105·3	—
4th day	3·11	51·4	3·5	—	92·1	—
6th day	3·33	55·0	3·4	—	89·5	—
8th day	3·49	57·7	3·4	2·8	89·5	73·69
16th day	3·78	62·5	2·9	2·9	76·3	76·30
21st day	3·82	63·1	2·8	2·9	73·69	76·30

TABLE IX

FIBRIN EREPTIC DIGEST

5 ml. of digest contains 61·35 mg. protein.

5 ml. of digest contains 7·51 mg. amino nitrogen = 5·046 ml. alkali.

13th day Tryptic Digest put on Erepsin.

Period of digestion.	Formol titre. ml.	Peptide split. %	Tryptophan liberated per cent.		Hydrolysis per cent based on alkaline hydrolysis value.	
			Aldehyde method.	Bromine method.	Aldehyde method.	Bromine method.
0·0	3·33	65·4	3·00	3·30	78·94	86·84
2nd day	3·63	71·9	3·20	—	84·20	—
4th day	3·77	74·7	3·62	—	95·26	—
7th day	3·89	77·0	3·66	—	96·32	—

Regarding the rate of liberation of tryptophan by activated papain hydrolysis, the following observations can be made. The three proteins, edestin, casein and blood fibrin were acted on by activated papain. The maximum peptide hydrolysis in the case of fibrin when digested for a period of thirty days was 50·7%. The apparent tryptophan value which was 102·7% on the 4th day, rose to 110·5% on the 5th, fell suddenly to 97·36% after which it rose to a maximum of 134·2% on the 9th day and gradually diminished to 113·2% on the 17th day and the same value was obtained on the 30th day. The free tryptophan was only 19·73% on the 14th day, rose to 34·21% on the 17th and the value obtained on the 30th day was 43·15%. Here again, the apparent tryptophan value could only be due to highly chromogenic polypeptides and the tryptophan was split off only slowly in parallel with the splitting of amino groups. The results are presented in Tables 10 to 12.

TABLE X

CASEIN PAPAIN DIGEST

5 ml. of the digest contains 96·6 mg. of protein.
5 ml. of the digest contains 10·65 mg. amino nitrogen = 7·16 ml. alkali.

Period of digestion.	Formol titre. ml.	Increase in titre. ml.	Peptide split. %	Tryptophan liberated per cent.	
				Aldehyde method.	Bromine method.
0·0	0·40	—	—	—	—
1st day	2·50	2·10	29·33	—	—
2nd day	2·77	2·37	33·10	1·56	—
3rd day	2·87	2·47	34·49	1·65	—
4th day	2·93	2·53	35·34	1·89	—
5th day	3·10	2·70	37·71	1·67	—
6th day	3·19	2·79	38·96	1·65	—
9th day	—	—	—	1·63	0·64
10th day	—	—	—	1·60	—
12th day	3·20	2·80	39·11	1·60	0·67
13th day	—	—	—	—	0·70
15th day	—	—	—	—	0·87
28th day	3·86	3·46	48·33	1·60	0·90

TABLE XI

EDESTIN PAPAIN DIGEST

5 ml. of digest contains 85·65 mg. protein.
5 ml. of digest contains 9·50 mg. amino nitrogen = 6·42 ml. alkali.

Period of digestion.	Formol titre. ml.	Peptide split. %	Tryptophan liberated per cent.		Hydrolysis per cent based on alkaline hydrolysis value.	
			Aldehyde method.	Bromine method.	Aldehyde method.	Bromine method.
0·0	0·46	—	—	—	—	—
5th day	2·86	37·4	1·50	—	107·2	—
6th day	2·90	38·0	1·62	—	115·7	—
8th day	2·96	38·9	1·65	0·70	117·9	50·00
11th day	—	—	—	0·74	—	52·85
22nd day	3·26	43·6	1·76	1·20	125·7	85·72

TABLE XII

FIBRIN PAPAIN DIGEST

5 ml. of digest contains 82·5 mg. protein.
5 ml. of digest contains 10·28 mg. amino nitrogen = 6·902 ml. alkali.

Period of digestion.	Formol titre. ml.	Peptide split. %	Tryptophan liberated per cent.		Hydrolysis per cent based on alkaline hydrolysis value.	
			Aldehyde method.	Bromine method.	Aldehyde method.	Bromine method
0·0	0·36	—	—	—	—	—
3rd day	2·60	32·4	—	—	—	—
4th day	2·88	36·5	3·9	—	102·70	—
5th day	2·93	37·2	4·2	—	110·50	—
6th day	3·06	39·10	3·7	—	97·36	—
7th day	3·20	41·10	3·7	—	97·36	—
8th day	3·22	41·40	3·7	—	97·36	—
9th day	3·23	41·60	5·1	—	134·20	—
10th day	—	—	4·7	—	123·70	—
12th day	3·32	42·9	4·6	—	121·10	—
14th day	3·35	43·3	4·4	0·75	115·80	19·73
17th day	3·41	44·2	4·3	1·30	113·2	34·21
30th day	3·86	50·7	4·3	1·64	113·2	43·15

Casein was digested more rapidly by activated papain, the peptide hydrolysis became 29·33% on the 2nd day and rose to a value of 48·33% on the 28th day. The apparent tryptophan value which was 1·56% on the 2nd day reached a maximum of 1·89% on the 4th, the value gradually decreased to 1·60% on the 10th day and continued to remain constant till the 28th. The free tryptophan value was 0·64% on the 9th day and the value rose successively to 0·67% on the 12th, 0·70% on the 13th, 0·87% on the 15th, 0·90% on the 28th day. The tryptophan was split off more rapidly than in the case of fibrin indicating that the mode of linking of tryptophan in the casein molecule could be quite possibly different from that of fibrin.

The peptide split in the case of edestin rose to 37·4% on the 5th day and reached 43·6% on the 22nd day showing a similarity with the other two proteins. The apparent tryptophan value was 107·2% on the 9th day, rose to 125·7% on the 22nd day. Fifty

per cent of the tryptophan was in the free condition on the 8th day, increased to 52·85 per cent on the 11th day and reached 85·72% on the 22nd day. Papain liberates the maximum amount of tryptophan from edestin and the rate of liberation of tryptophan compares favourably with that of casein.

The three proteins under study differ markedly in their digestibility towards the enzymes. Thus in the case of casein and edestin nearly 75% of the total tryptophan was in the free condition even before the end of six hours of digestion when the peptide hydrolysis was only 40%. But only 42·5% of the tryptophan in fibrin was in the free condition at the end of 24 hours of digestion with a peptide hydrolysis of nearly 43·1%. The liberation was complete in the case of casein at the end of 4 days. Trypsin liberated only 93% in the case of edestin and 76% in the case of fibrin. Further digestion with intentinal erepsin liberated almost all the tryptophan from edestin and 96% in the case of fibrin. Thus in the case of fibrin, the tryptophan peptide linkage was not so easily susceptible to enzyme attack. This result agrees with the observations of Fürth and Lieben[3] in their work on the enzymic digestion of blood fibrin. Since an appreciable quantity of the tryptophan of casein and edestin appears in the free condition at quite an early period of digestion, it has to be concluded that this part of it at least occupies an exposed position in the protein molecule. Unless work is extended to a number of proteins, no generalisation can be drawn regarding the position of the amino acid in the protein molecule and its hydrolytic splitting by the enzymes of the digestive tract and by activated papain.

Summary

The liberation of tryptophan during the successive action of pepsin, trypsin and erepsin on the three different proteins casein, edestin and blood fibrin has been studied in detail. The percentage peptide split, the apparent tryptophan value as obtained by the aldehyde method and the value for 'free' tryptophan by the new bromine method have all been followed throughout the series of enzymic digestions. Similar studies have been carried out on the three proteins with activated papain. The results obtained have been discussed with reference to the chromogenic value of tryptophan containing peptides as distinguished from the free amino acid and to the position of the tryptophan residue in the three proteins.

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BIBLIOGRAPHY

1. Hopkins, F. G. and Cole, S. W., *J. Physiol,* 1901, *27,* 418.
2. Abderhalden, E. and Reinbold, R., *Hoppe-Seyl. Z.,* 1905, *46,* 166.
3. Fürth, O. and Lieben, F., *Biochem, Z.,* 1920, *109,* 103.
4. Vanslyke, D. D., Hiller, A., and Dillon, R. T., *J. Biol. Chem.,* 1942, *146,* 137.
5. Ragins, I. K., *J. Biol. Chem.,* 1928, *80,* 544.
6. Onslow, H., *Biochem. J.,* 1921, *15,* 383.
7. Northrop, H., *Jour. Gen. Physiol.,* 1930, *13,* 739.
8. Calvery, H. O., *J. Biol. Chem.,* 1936, *113,* 21.
9. Bergmann, M., *J. Biol. Chem.,* 1937, *118,* 781.
10. Abderhalden, E., *Fermentforschung,* 1937, *15,* 314.
11. Neuberg, C., *Biochem Z.,* 1910, *24,* 441.
12. Abderhalden, R., *Ferentforschung,* 1937, *15,* 306.
13. Fearon, W. R., *Nature,* 1948, *162,* 338.
14. Greenhut, I. T., Schweigert, B.S., and Elvehjem, C. A., *J. Biol. Chem.,* 1946, *165,* 325.
15. Horn, M. J. and Jones, D. B., *J. Biol. Chem.,* 1945, *157,* 153.
16. Cohn, E. J. and Hendry, M., *Organic Synth.,* 1930, *10,* 101.
17. Damodaran, M. and Anantanarayanan, P., *Biochem. J.,* 1938, *32,* 1888.
18. Damodaran, M. and Krishnaswamy, T. K., *Proc. Indian Acad. Sci.,* 1942, *15,* 285.
19. Bates, R. W., *J. Biol. Chem., 119,* 1937.
20. Plimmer, R. H. A., *The Chemical Constitution of the Proteins,* Part I, p. 132, Longmans Green & Co., London.
21. Shaw, J. L. D. and McFarlane, W. D., *J. Biol. Chem.,* 1940, *132,* 387.

NOTE

With reference to my paper 'Two new species of *Irona* (Isopoda) parasitic on Madras fishes' published in Vol. No. XX, Pp. 66-74 of this Journal I wish to state that Mrs. Florence Abraham had been working on the same species in the same laboratory when she was research assistant from November 1947 to February 1950 and had made prior observations on the subject. Therefore a fuller systematic account of parasitic isopods may be published by her later.

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REVIEW

We have received the first issue of the Journal of Scientific Research of the Benares Hindu University Vol. I, 1950-51. The Journal is published by the Benares Hindu University and is edited by Dr. R. K. Asundi, University Professor of Spectroscopy, Benares Hindu University. It contains original papers on several aspects of both physical and biological sciences. We wish the Journal every success.

EDITOR.

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